THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

JOINT MEETING

A JOINT Meeting of the Society with the Food Law Institute of the United States, the Food Group of the Society of Chemical Industry, and the Association of Public Analysts, of Great Britain, was held on Friday, July 26th, 1957, in the Lecture Theatre of the Royal Institution, 21 Albemarle Street, London, W.1. The meeting was the occasion of a Conference on The Control of Chemical Additives in Food. Over 390 members and visitors were present.

The subject for the morning session, which began at 10 a.m., was "The Control of Chemical Additives in Food, other than antibiotics." Dr. A. J. Amos, F.R.I.C., first introduced Mr. J. B. Godber, M.P., Joint Parliamentary Secretary for the Ministry of Agriculture, Fisheries and Food, who opened the Conference by welcoming the overseas visitors and expressed the keen interest of his Ministry and of the Ministry of Health and the Department of Health for Scotland in this vital subject. The President, Dr. J. H. Hamence, M.Sc., F.R.I.C., then introduced Mr. Charles Wesley Dunn, President of the Food Law Institute of the United States, who took the Chair for the session. Mr. Dunn expressed the great interest being shown by the United States Congress in the matter. The session consisted of papers by Mr. G. P. Larrick, Commissioner of Food and Drugs in the Food and Drug Administration of the United States Department of Health, Education and Welfare, and Dr. A. J. Lehman, Director of the Division of Pharmacology in the Food and Drug Administration of the United States Department of Health, Education and Welfare (presented on their behalf by Mr. J. L. Harvey, Deputy Commissioner of Food and Drugs in the Food and Drug Administration of the United States Department of Health, Education and Welfare); Dr. C. A. Morrell, Director of the Food and Drug Directorate in the Canadian Department of National Health and Welfare (presented on his behalf by Dr. B. L. Oser, Director, Food Research Laboratories Inc., New York); Dr. N. C. Wright, C.B., Chief Scientific Adviser (Food) to the Ministry of Agriculture, Fisheries and Food; and Dr. E. J. Miller, Chemist in the Plant Pathology Laboratory, Ministry of Agriculture, Fisheries and Food. A general discussion followed.

The subject for the afternoon session, which began at 2.30 p.m., was "The Control of Chemical Additives in Food with Special Reference to Antibiotics." The Chair was taken by Sir Harry Jephcott, M.Sc., Hon. D.Sc., F.P.S., F.R.I.C., Chairman of the Council of the Department of Scientific and Industrial Research. The session consisted of papers by Dr. H. Welch, Director of the Division of Antibiotics in the Food and Drug Administration of the United States Department of Health, Education and Welfare (presented on his behalf by Mr. J. L. Harvey); Dr. C. A. Morrell and Dr. F. S. Thatcher, Chief of the Microbiology Laboratory in the Food and Drug Directorate of the Canadian Department of National Health and Welfare (presented on their behalf by Mr. C. A. Adams); and Mr. A. L. Bacharach, Chairman of the *Sous-Commission* on Antibiotics in Food appointed by the *Commission Internationale des Industries Agricoles* and the *Bureau International Permanent de Chimie Analytique*. Dr. B. L. Oser opened the discussion that followed with a prepared contribution.

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

THE following is a summary of the paper presented at the Joint Meeting of the North of England Section and the Physical Methods Group on Friday, October 19th, 1956, in Manchester, by T. S. Work, Ph.D., D.Sc., entitled "Some Recent Applications of Ion Exchange in Biochemistry." A first report appeared in *The Analyst*, **1956**, **81**, 678.

Some Recent Applications of Ion Exchange in Biochemistry

By T. S. Work

Biochemists have as their problem the chemistry of the whole variety of living things. Perhaps it is for this reason that new analytical techniques frequently find their first and most enthusiastic supporters among biochemists. Biochemical research tends to be limited not by abstract thought but rather by the available methodology and, during the past 15 years, that methodology has expanded in the most remarkable fashion. The introduction of radioactive isotopes, of paper chromatography and of ion-exchange chromatography is of particular value and interest to biochemists, because it enables us at last to make some real impression on the chemical problems involved in handling proteins and nucleic acids. In considering the application of ion-exchange methods to biochemistry, it is convenient to divide the subject into three sections, one dealing with the isolation and purification of large macromolecules with molecular weights over 10,000, a second covering the problems involved in fractionation of complex mixtures of polypeptides or nucleotides and a third concerned with methods used for the accurate analysis of these compounds.

Fractionation of macromolecules

In the fractionation of either proteins or nucleic acids, a severe limitation is imposed by insolubility and instability of these large molecules in non-aqueous systems. Most proteins and nucleic acids are, however, soluble in dilute buffer solutions and all have ionisable groups, so that ion-exchange methods of fractionation should be generally applicable.

Sulphonated polystyrene resins, such as Zeo-Karb 225 or Dowex 50, have been tried in protein fractionation by Boman,[1] but the protein cannot penetrate the resin beads and the capacity is low.

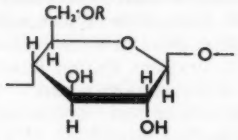
A preliminary report has appeared also on the use of Dowex 2, a strongly basic quaternary ammonium polystyrene resin, for the fractionation of plasma proteins. The buffer used was 2-amino-2-hydroxymethylpropane-1:3-diol and the column loading was 0·5 ml of serum on a 18-cm × 1·5-cm column. This is a low loading and thus a disadvantage for large-scale work, but the resin seems to be suitable for the isolation of small amounts of enzymes from crude mixtures. Boman[2] reported that serum albumin, prostatic phosphatase, haemoglobin, phycoerythrin and phycocyanin all gave narrow well defined zones on his columns.

Considerable success has been reported with the cation-exchange resin Amberlite IRC-50, an acrylate resin possessing free carboxyl groups. This resin has been most successful in the fractionation of basic proteins over the pH range 6·0 to 7·5. The finely divided resin is equilibrated with sodium phosphate buffer solution and the protein, dialysed against the same buffer solution, is then put on top and the column is developed with buffer solution (of increasing molarity when necessary) without change in pH. Using this method, Hirs[3] was able to isolate chymotrypsinogen (molecular weight, 22,000; isoelectric point, 9·1) from an extract of dried pancreas powder, and Hirs, Moore and Stein[4] were able to isolate pure ribonuclease, a basic protein of molecular weight about 12,000, from the same source. Paléus and Neilands[5] successfully purified cytochrome *c* (molecular weight, 13,000; isoelectric point, 10·1) by a similar method. Lysozyme (molecular weight, 15,000; isoelectric point, 10·5) was also successfully purified on Amberlite IRC-50 by Tallan and Stein.[6]

It should be noted that for each of these proteins the isoelectric point is high and, by protein standards, the molecular weight is low. It is reasonable to assume that the carboxyl group of the resin holds the basic groups of these proteins by electrostatic forces. As might be expected from this assumption, the elution rate is increased either by increasing ionic strength or by raising the pH towards the isoelectric point of the protein. In the first instance, the inorganic cation displaces the protein cation from the resin, in the second, the availability of protein cations is reduced. It might be supposed that, if the pH was lowered sufficiently to reduce ionisation of the resin carboxyl groups, the rate of elution would increase again, and in fact with lysine as a test substance columns of Amberlite IRC-50 behave as might be expected from theoretical considerations. With proteins, however, the situation seems to be more complex, since, on lowering the pH of the column below 5·0 with either lysozyme or cytochrome *c*, Boardman and Partridge[7] found that the protein band was strongly adsorbed at the top of the column and could not be eluted without raising the pH.

Some success has been obtained by using Amberlite IRC-50 for fractionating larger proteins of lower isoelectric point; for example, some haemoglobins have been successfully

fractionated by Partridge, but it seems likely that a new group of ion-exchange materials, prepared from cellulose by Peterson and Sober,[8] are likely to become more important for the fractionation of neutral or acidic proteins. Four types of modified cellulose have been studied, carboxymethylcellulose prepared from Whatman paper powder with sodium hydroxide and chloroacetic acid, a cellulose phosphate prepared by treating paper powder with phosphorus oxychloride, both of these providing cation-exchange powders, and two anion-exchange powders made by treating paper powder or Solkafloc with either epichlorhydrin, triethanolamine and sodium hydroxide or with diethylaminoethyl chloride and sodium hydroxide. The methods of preparation of these cellulose ion-exchange resins are empirical, and so far no study has been made of the chemical nature of the products, but from the conditions used it may be assumed that the products are cellulose ethers or, in the phosphorylated product, a cellulose ester. The amount of reagent used is such that the final acid or base content of the cellulose is about 1 milli-equivalent per g of cellulose; there is thus considerably less than one ether linkage per glucose unit and the general structure is probably of the form—



where *R* occurs about once in every five glucose residues. The material tried most extensively is the diethylaminoethyl derivative, which has been used successfully for the fractionation of plasma proteins both in the laboratory and on a commercial scale; thus, for example, horse serum has been successfully fractionated by Sober, Gutter, Wycoff and Peterson.[9] Diethylaminoethylcellulose has also been used successfully in the fractionation of γ-globulins (Porter, unpublished observations).

Diethylaminoethylcellulose differs from Amberlite IRC-50 in that, being an anion-exchange resin, it is more suitable for neutral or acidic proteins. It has a very large capacity, loads of up to 25 mg of protein nitrogen per g of powder being well resolved. This seems to be about 25 times the loading that can be used with Amberlite IRC-50. The diethylaminoethylcellulose responds to change in pH and ionic strength in the manner to be expected from theoretical considerations. Maximum capacity is obtained with low ionic strength. The rate of travel of the protein bands is increased with increasing strength of inorganic anion, and the usual procedure is to hold the pH constant and to increase steadily the concentration of sodium chloride or phosphate buffer. Decreasing pH decreases the anionic character of the protein, and increasing pH reduces the ionisation of the tertiary amino groups of the adsorbent, so that both changes also tend to increase the rate of travel of protein through the column.

The other modified celluloses have not yet been applied to any extent in the analysis of protein mixtures, but preliminary experiments reported by Sober and Peterson[10] suggest that they will have wide application. The anion-exchange resin made by reaction of cellulose with epichlorhydrin and triethanolamine seems promising for the analysis of deoxyribonucleic acids and ribonucleic acids (Bendich, Fresco, Rosenkranz and Beiser[11]), but so far only preliminary results have been published. Any method likely to achieve analysis of these acids should assume great importance, since these high polymers have not so far been successfully fractionated by any available method and their biochemical interest and importance are immense.

It is clear from all this preliminary work that ion-exchange methods both for the analysis of protein mixtures and for the isolation of pure proteins and nucleic acids are likely to assume considerable importance during the next 10 years.

Peptides and polynucleotides

The larger naturally occurring peptides such as insulin, hypertensin, oxytocin, vasopressin, adrenocorticotrophic hormone, intermedin, bacitracin and bradykinin, present much the same problems in purification as do the smaller and more stable proteins. Relatively little use has been made of ion-exchange methods to purify these compounds, but this is probably mainly because counter-current distribution or ionophoresis have generally been

sufficient. Ion-exchange methods can be most successful, as shown by the fractionation of vasopressin and oxytocin on Amberlite IRC-50 by Taylor.[12]

Ion-exchange chromatography has, however, been outstandingly successful in the fractionation of complex mixtures of peptides obtained by partial hydrolysis of proteins and in separating the smaller polynucleotides from enzymic digests of ribonucleic acids. For this type of separation, the requirements are rather different from those involved in fractionation of proteins or nucleic acids. The products of partial hydrolysis are chemically stable and can, therefore, be handled over a wide range of pH values. To take advantage of this, it is necessary to use strongly acidic or strongly basic ion-exchange resins, *i.e.*, sulphonic acid or quaternary ammonium resins, since the weakly acidic or weakly basic resins can only be used over a narrow pH range without suppressing the ionisation of their reactive centres. Zeo-Karb 225, Dowex 50 and Amberlite IR-120, all polystyrenesulphonic acids, are suitable for fractionation of peptides over the pH range 2 to 7. For good results, the degree of cross linking should be somewhere within the range 2 to 5 per cent. of divinylbenzene. Below 2 per cent. of divinylbenzene, the resins tend to become rubbery and mechanically unsuitable for columns, whereas above 5 per cent. of divinylbenzene larger peptides may tail (Campbell and Work[13]). Askonas, Campbell, Godin and Work[14] obtained particularly sharp fractionation of a complex peptide mixture by gradient elution from a column of Zeo-Karb 225 (water regain, 1·55; about 5 per cent. of cross-linking agent). When small traces of peptides have to be isolated from large volumes of effluent, it is a considerable advantage to use volatile buffers—in this instance ammonium formate.

A similar type of fractionation, but of a partial hydrolysate of ribonucleic acid, has been carried out by Volkin and Cohn,[15] who used an anion-exchange resin to separate the various acidic components. Here also the degree of cross linking has to be kept low, and Dowex 1×2 (about 2 per cent. of cross-linking agent) was found most suitable. The column was developed with steadily decreasing pH, and with increasing ionic concentration (Cl').

Quantitative analyses of amino acids

Finally, cation-exchange resins have been used for the quantitative study of protein composition, mainly by Moore and Stein.[16] The problem is to achieve complete separation of all individual amino acids in a complete protein hydrolysate; to do this a rather long column is required (150 cm). Successful results can be obtained with sulphonated polystyrene resins varying in cross linkage from 4 to 10 per cent., but, as the degree of cross linking rises, the displacement of basic amino acids becomes progressively slower. Accurate control of pH and temperature is imperative, a change of a few degrees making all the difference between complete and incomplete resolution. Jacobs[17] has found that reliability of the colour reaction is much improved if the ninhydrin is first treated with an ion-exchange resin to remove trace-metal impurities. This method generally involves a rather tedious colorimetric analysis of 600 individual samples, but Moore and Stein (unpublished observations) have now devised a fully automatic method of analysis of column effluents, which enables the complete analysis of a protein to be carried out in 48 hours.

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Obituary

GERALD ROCHE LYNCH

GERALD ROCHE LYNCH died on Wednesday, July 3rd, at the age of 68. By his death many scientific and other organisations lost a prominent figure, for he had occupied many presidential chairs. Unlike many other leading scientists, his name was well known to the public as one of the central witnesses for the prosecution in many famous murder trials. Primarily a medical man, he was also an eminent analytical chemist and a great authority in the field of toxicology.

Roche Lynch, O.B.E., M.B., B.S., D.P.H., F.R.I.C., L.M.S.S.A., was born on January 12th, 1889, the son of Dr. Jordan Roche Lynch of Notting Hill. He was educated at St. Paul's School, and entered St. Mary's Hospital around 1906; indeed, this hospital became his life, although he worked under Professor H. E. Armstrong for two years (1908–10) at the City and Guilds Institute. He served as a medical man in the Navy in the 1914–18 war. Working under Sir William Willcox, he became Assistant Chemical Pathologist at St. Mary's in 1920. He was lecturer in forensic medicine and toxicology at Westminster Hospital from 1924 to 1945, and took a similar position at St. Mary's in 1934, occupying this post until his retirement in 1954.

His association with the Home Office, and hence with the Criminal Investigation Department of Scotland Yard, commenced in 1920 when he was appointed Assistant Official Analyst. He became Senior Official Analyst in 1928, and occupied this position until 1954. From 1920 to 1954 there was scarcely an important murder trial, especially those in which poison was involved, in which he was not one of the principal witnesses for the prosecution. In 1930 he was the centre of the prosecution in no less than three poison trials, the results of which were the hanging of three women. Another famous case in which he was involved was the murder of Police Constable Gutteridge, for which Browne and Kennedy paid the supreme penalty. In the field of toxicology there were few *causes célèbres* in which he was not involved. As a witness he was scrupulously fair, but firm under cross-examination, and one of the nicest compliments paid him was by the present Lord Chief Justice when he was the guest of the Royal Institute of Chemistry at the annual dinner given in the year Roche Lynch retired as President.

Roche Lynch was essentially a quiet man, and he refused considerable sums of money from publishers who chased him for his memoirs based on his case books. Although others might have been tempted, such a step never appealed to him, as all who knew him well will realise.

His main scientific loves were the Medico-Legal Society (he was their oldest Past President), the Royal Institute of Chemistry, of which he was President (1946–49), and our own Society. He was Honorary Secretary of the Society of Public Analysts and Other Analytical Chemists (1932–36), a member of Council (1929–30 and 1938–50), a Vice-President (1931) and President (1936–37). In other fields he was equally as eminent, being Master of the Society of Apothecaries of London (1951–52), and, as a keen freemason, he was a Grand Lodge Officer belonging to the R.N.V.R. Lodge No. 3923 and Grand Master's Lodge No. 1.

So much for his brilliant career. As a man, he held, and did not hesitate to express, strong opinions, being far from a courtier. Nobody could be blunter, but he was rarely wrong in his appraisal of people or situations. But there was always a very understanding and gentle side to his nature. As an examiner in Branch E of the Royal Institute of Chemistry, there was no one fairer or more understanding, in spite of his gruff manner. He was a great lover of music, as well as a keen and successful gardener. He was rarely seen without a carnation, always home grown. His other interest was collecting old clocks, and on these he was a recognised authority. Amongst others—he had scores—he had a Tompion, a Graham and a Quare.

Once he had decided that he liked you—and that was only after a searching trial—he was the staunchest of friends; but that did not mean that you were immune from criticism. He was untouched by flattery or by the normal glittering prizes of life. He just remained Roche Lynch, unperturbed by success or failure.

He married Sybil Marguerite Pinnock in 1919, and she died at the early age of 29. He leaves behind his daughter Bridget (Mrs. David), so well liked by her many friends in the chemical world.

D. W. KENT-JONES

Some Experiments with Spectrofluorimeters and Filter Fluorimeters

By C. A. PARKER and W. J. BARNES

(*Presented at the meeting of the Society on Wednesday, April 3rd*, 1957)

Fluorescence emission spectra of liquids have been measured by using laboratory-made accessories in conjunction with a small monochromator, photomultiplier tube and commercially available a.c. amplifier. The apparatus can also be used for the measurement of fluorescence "excitation" spectra, or as a simple filter fluorimeter when high sensitivity is required. Examples of the analytical application of fluorescence emission spectra are given. A Dewar vessel for the measurement of low-temperature luminescence or absorption is also described.

Some general considerations, of particular importance in quantitative work, include (*a*) the wavelength of the exciting light and its intensity, (*b*) photo-decomposition, (*c*) inner filter effect, (*d*) reversible quenching and irreversible oxidation by molecular oxygen and (*e*) temperature. These are discussed in relation to the system $R + X \rightleftharpoons RX$, in which the concentration of X is measured by the addition of a reagent to give the fluorescent compound RX. Examples are taken from work recently carried out on the reaction of borate with benzoin.

Simple chemical actinometry is recommended for the measurement of exciting intensities, particularly when instrumental sensitivities are to be quoted, or relative sensitivities with different exciting wavelengths have to be determined.

With the advent of the photomultiplier tube the measurement of fluorescence intensities at very low levels has become commonplace, and, in principle, fluorimetric analysis can be carried out at exceedingly minute concentration. By the use of a spectrometer to disperse either the exciting or fluorescent light, the selectivity of the method can be greatly increased, the loss of sensitivity due to the limited aperture of the spectrometer being offset either by amplification of the output from the photomultiplier tube before detection or by increase in the intensity of the exciting light. In practice, of course, the sensitivity is generally limited by factors such as irrelevant fluorescence from reagents or cuvette, inefficiency or fluorescence of filters, or by photo-decomposition.

The experiments to be described were carried out to obtain some preliminary information about the analytical possibilities of fluorescence spectrometry and to investigate the importance of some of the limiting factors. For the latter, a typical problem in trace analysis was selected, namely the determination of sub-microgram amounts of boron by reaction with benzoin to produce a fluorescent compound.[1]

Apparatus

Depending on the nature of the problem, it may be necessary to use either filters or a spectrometer for either the exciting or the fluorescent light. The apparatus was therefore kept as versatile as possible. The light sources, spectrometers, detectors and cell compartment were separate units that could be readily linked together in one way or another as required. For the same reason a direct-reading rather than a null-point method was used; the output from the amplifier was measured with a multi-range meter or fed to a 2-mA pen recorder. By stabilisation of the mains supply to the amplifier and mercury-vapour lamp, and by using dry batteries for the H.T. supply to the photomultiplier tube, fluctuations in output were kept within acceptable limits (less than 1 per cent.). The fluorescence intensity was measured in terms of that obtained with a suitable concentration of a standard (quinine sulphate in 0·1 *N* sulphuric acid) contained in a comparison cell.

Cell compartment—

A plan of the cell compartment is shown in Fig. 1 and it is shown in Figs. 2 and 3 set up with a Hilger small quartz double monochromator (D222/D205) for the measurement

of fluorescence emission spectra. It consists simply of a box with four apertures, and a vertical slide by which either of the two silica cells containing the standard or test solutions can be moved into the beam. The cells are 15 mm × 35 mm and 45 mm high and require 15 ml of solution. They have loosely fitting lids and provision is made for de-aeration by means of a current of nitrogen. The sample can be illuminated by chopped light either direct from the mercury-vapour lamp at A or from a monochromator at F. Fluorescence is detected either via the monochromator at E or (for high sensitivity) directly by the photomultiplier tube at G.

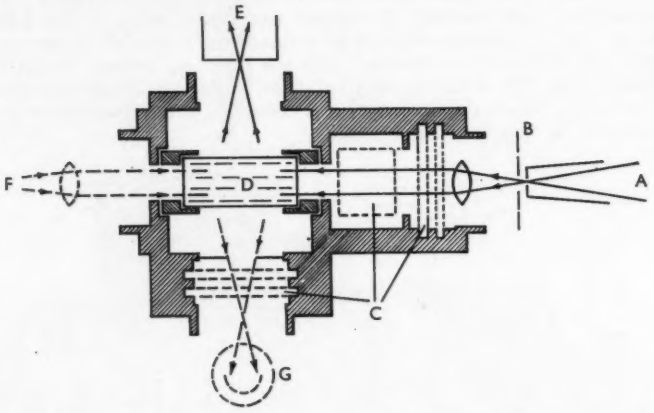

Fig. 1. Cell compartment for spectrofluorimeter or filter fluorimeter: A, light from mercury-vapour lamp; B, chopper disc; C, glass and liquid filters; D, silica cell in vertical slide; E, monochromator for fluorescent light; F, chopped exciting light from monochromator; G, photomultiplier for filtered fluorescent light. For absorption measurements, G is replaced by a light source. For monitoring exciting light from A, F is replaced by a vacuum photo-tube

LIGHT SOURCES—

For most work, other than the measurement of fluorescence "excitation" spectra, a 125-watt high-pressure mercury-vapour lamp (type MB/D with glass envelope removed) has been found to be very convenient and satisfactory. To obtain maximum stability of light intensity, the lamp is housed in a closed water-cooled tube. A constant-voltage transformer has been found to give a sufficiently stable supply for most purposes, but operation from accumulator cells via a ballast resistor is ideal. Other types of mercury lamp have also been investigated and their characteristics are discussed later. For the measurement of "excitation" spectra, either a hydrogen lamp or, better, a xenon arc lamp must be used.

DETECTORS—

The output from the photomultiplier tube is passed to a Mervyn spectrometer amplifier (800-cycle), the chopper disc of which is inserted in the beam of exciting light (see Fig. 3). The amplified output is either read directly or fed to a recorder. Chopping the exciting light has the advantage that slight leakage of light into the cell compartment is of no consequence. If long-lived phosphorescence has to be measured, then the chopper must, of course, be placed in the beam of fluorescent light.

USE FOR ABSORPTION MEASUREMENTS—

The cell compartment is used directly for measurement of absorption spectra by placing a suitable source of continuous light at G (Fig. 1). Absorption of exciting light from A can be measured simply by means of a vacuum photo-tube (connected to a galvanometer) placed at F. This photo-tube can also be used for monitoring the intensity of the exciting light when, for example, photo-decomposition at various intensities is being investigated.

EXCLUSION OF OXYGEN—

An arrangement with a loosely fitting lid on the cuvette and a delivery tube for the nitrogen gas was found to provide sufficiently good de-oxygenation for many purposes. However, with compounds showing strong oxygen quenching, *e.g.*, the borate - benzoin compound, sufficient oxygen diffused under the lid to cause an objectionable error, even while a rapid stream of nitrogen was passing. If the gas stream was stopped momentarily, errors of 50 per cent. or more were observed. The special cell shown in Fig. 4 was therefore constructed; it allows the two reagents to be completely de-oxygenated before mixing and the fluorescence of the mixture to be measured under anaerobic conditions. After bubbling through the solution in the cuvette, A, the nitrogen passes through the delivery tube, B, the lower end of which is closed and acts as a liquid-tight stopper to prevent the reagent in the trap from flowing into the cuvette. The gas bubbles through the liquid in the trap from a small hole in the side of the delivery tube and finally vents through the annular space at the top of the trap. When de-oxygenation is complete, the delivery tube is lifted slightly to allow the solution to flow into the cuvette.

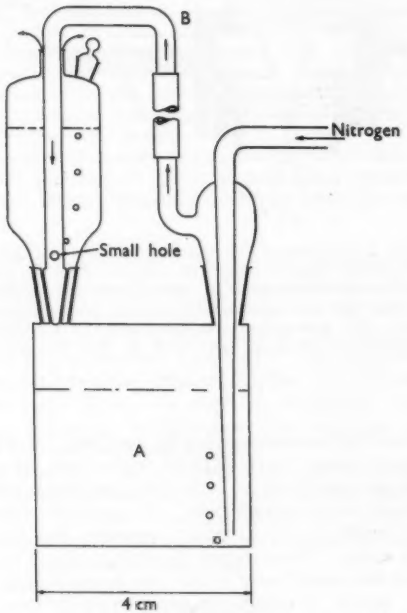

Fig. 4. Cell for reaction and measurement of fluorescence in the absence of oxygen

TEMPERATURE CONTROL—

When the fluorescent component takes part in equilibria in the solution, quite large temperature coefficients of fluorescence can be obtained.

The fluorescence of the solution of the borate - benzoin compound, for example, showed a decrease of 3 to 4 per cent. per degree rise in temperature. A constant-temperature block for the fluorimeter cells was therefore used in investigating this reaction. This consists of a hollow brass block that fits closely round the back and sides of the fluorimeter cells. The exciting light enters the cells from below. Fig. 5 shows the special cell for anaerobic measurements, together with an ordinary cell for the quinine sulphate standard solution, in position in the constant-temperature block. The block in its slide is placed in a simple light-tight housing in front of the spectrometer slit (see Fig. 6). In this case it was found more convenient to chop at the entrance slit of the spectrometer.

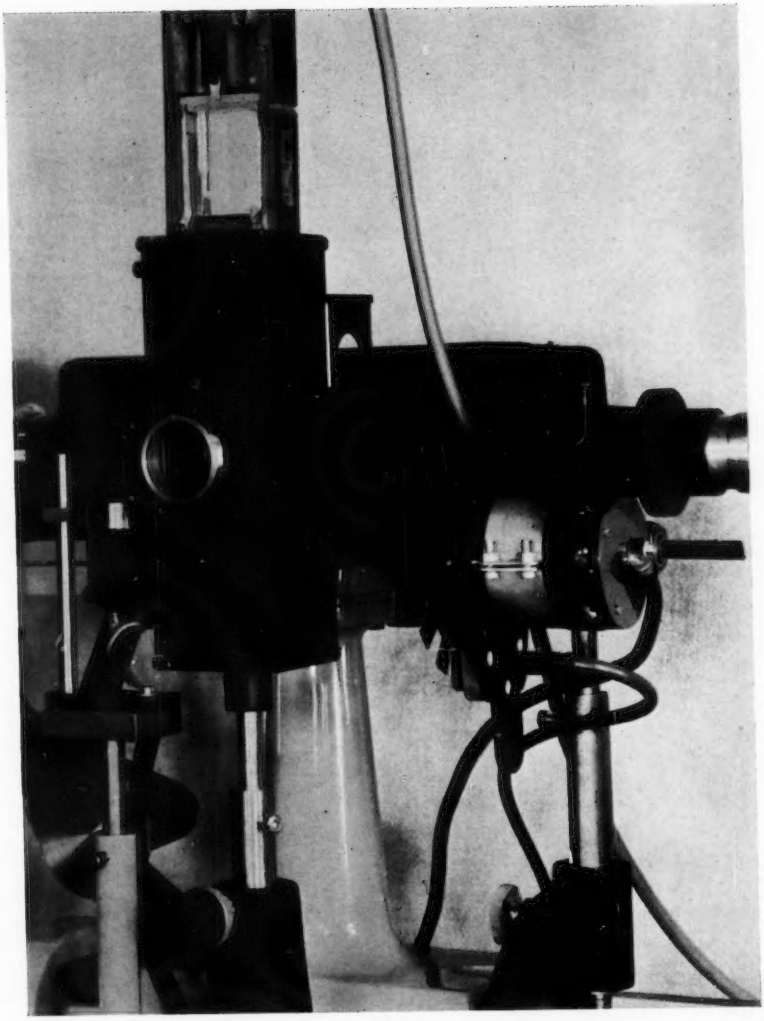

Fig. 2. Cell compartment, showing cell holder raised for removal of the top cell

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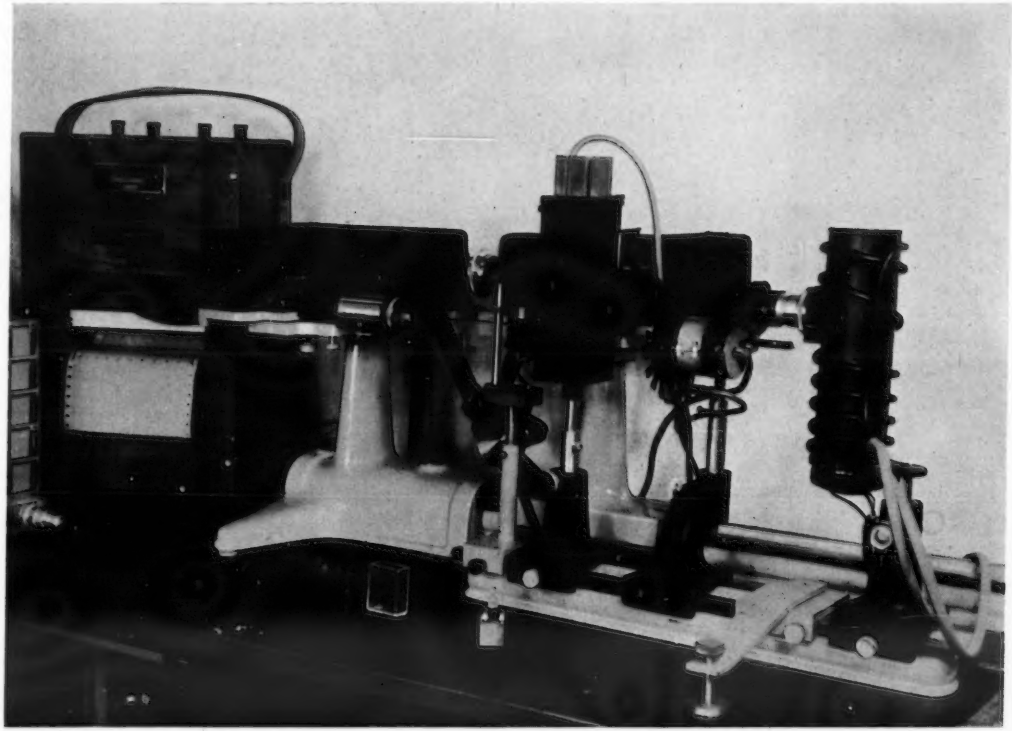

Fig. 3. Arrangement for the measurement of fluorescence emission spectra

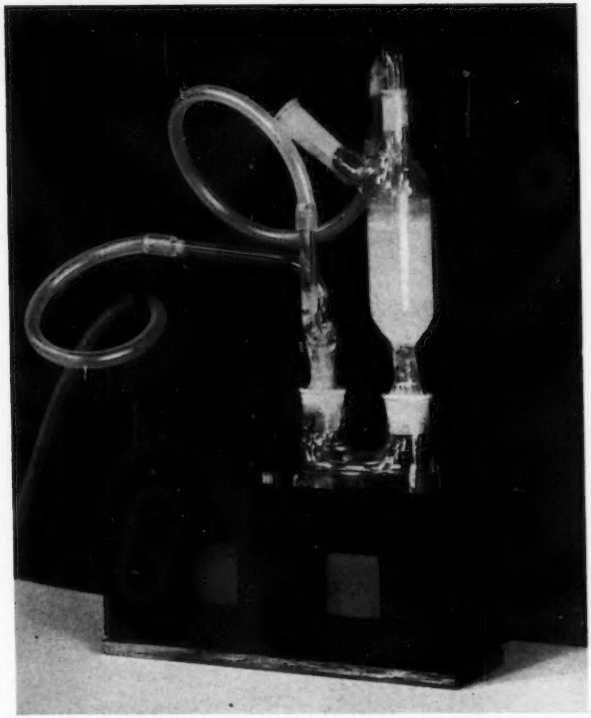

Fig. 5. De-oxygenation cuvette in constant-temperature block

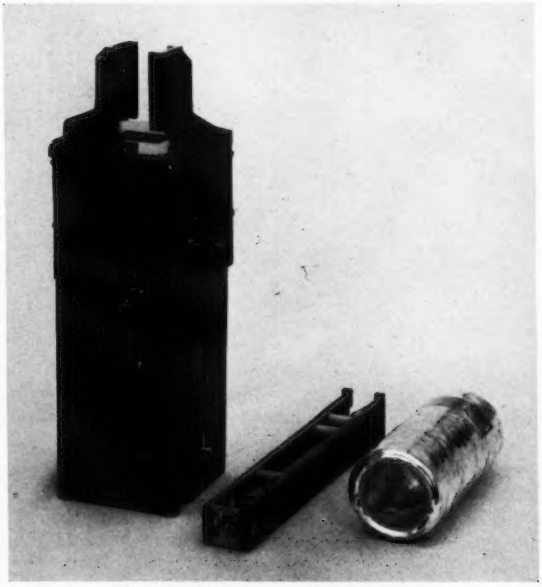

Fig. 7. Low-temperature apparatus

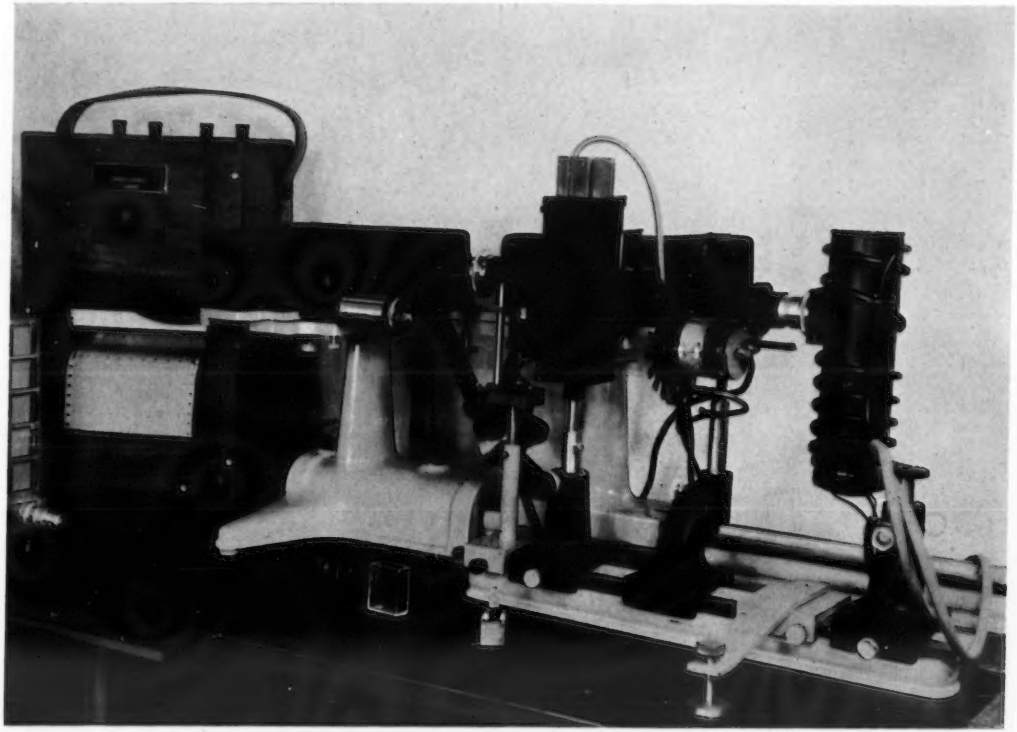

Fig. 3. Arrangement for the measurement of fluorescence emission spectra

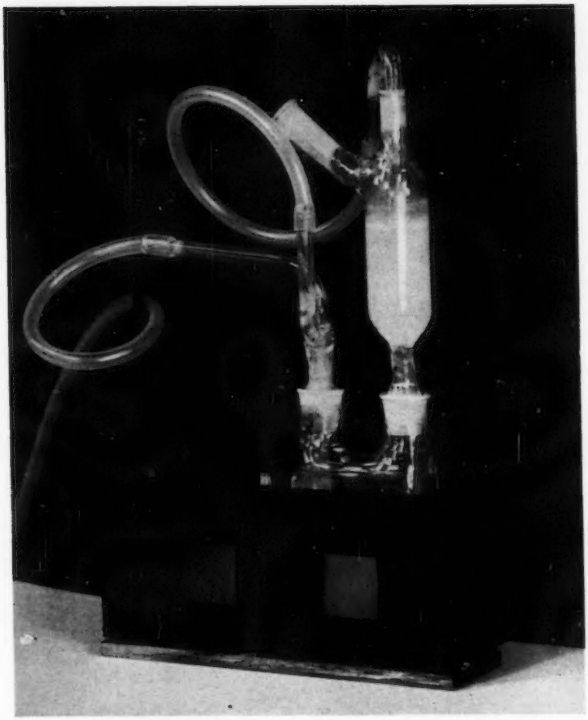

Fig. 5. De-oxygenation cuvette in constant-temperature block

Fig. 7. Low-temperature apparatus

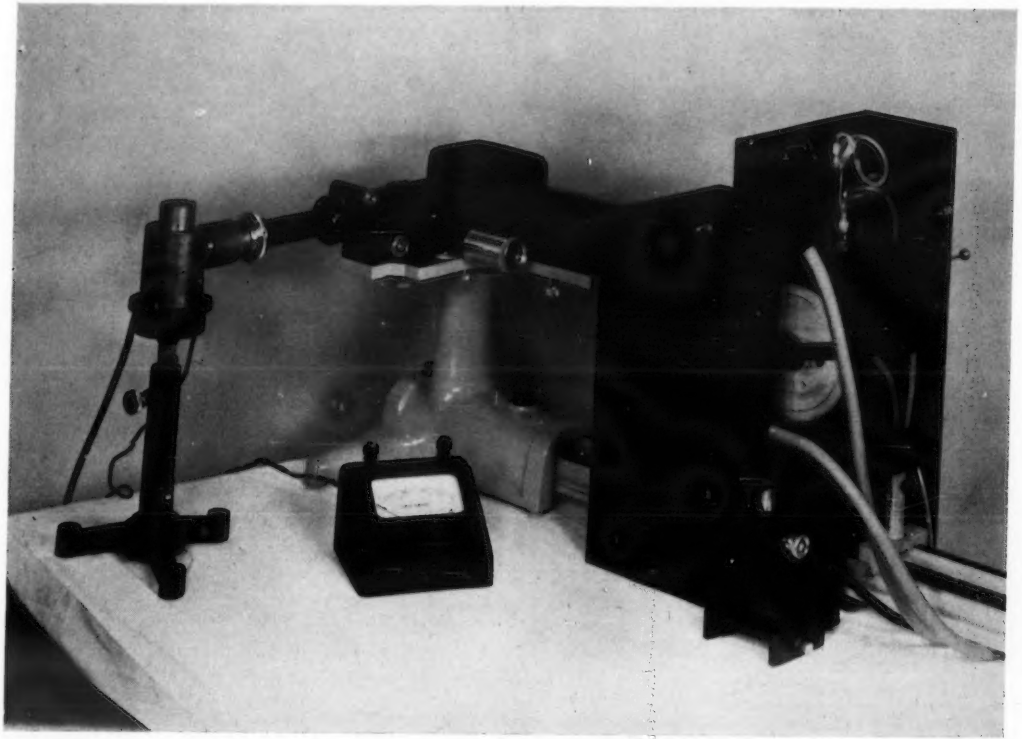

Fig. 6. Apparatus used for investigating the borate - benzoin reaction

DIRECT GALVANOMETER READING—

If the fluorescent compound can be separated completely from all other fluorescing substances, it is often satisfactory to use filters for the isolation both of the exciting and fluorescent light. The intensities then available are often sufficiently great to make the use of the chopper - amplifier unnecessary and the output from the photomultiplier tube may be read directly with a "spot" galvanometer (with, *e.g.*, 1 μA for full-scale deflection). For example, such a simple fluorimeter can be used for the determination of borate (after chemical separation) at very low concentration (less than 0·0001 μg per ml).

LOW-TEMPERATURE APPARATUS—

This is shown in Fig. 7; it consists of a silica Dewar vessel with plane windows seated in a tin box fitted at the top with guides in which slides the holder for the test and blank cells. The substance to be examined is dissolved in a suitable solvent mixture that freezes to a clear glass when immersed in liquid nitrogen. The apparatus can be used for both absorption measurements (with the Beckman spectrophotometer) and for fluorescence measurements, and is similar to that described by Norman and Porter.[2]

MEASUREMENT OF FLUORESCENCE EMISSION SPECTRA

The spectral sensitivity of the apparatus was determined by making measurements of photomultiplier output as a function of wavelength when a standard lamp run at a known colour temperature was placed in line with the entrance slit. The spectral sensitivity curves calculated from such results are shown in Fig. 8. They represent the relative photomultiplier response that would be obtained if measurements were made with a source of uniform spectral intensity, *i.e.*, constant number of quanta per unit interval of frequency. With both instruments the sensitivity falls off rapidly above about 525 mμ, owing to decreasing sensitivity of the photomultiplier tube, and for measurements above about 550 mμ a red-sensitive detector would have to be used. The glass-prism instrument is not suitable for measurements below about 390 mμ, owing to absorption by the prism material.

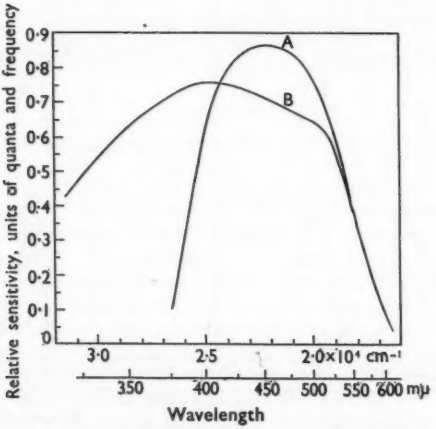

Fig. 8. Spectral sensitivities: curve A, "D 186" glass-prism constant-deviation spectrometer with RCA 931A photomultiplier tube; curve B, D222/D205 quartz double monochromator with Mazda 27M3 photomultiplier tube

The fluorescence emission spectra of some solutions as measured with the quartz monochromator are shown in Fig. 9 together with the corresponding absorption spectra. The two N-phenylnaphthylamines, commonly used as rubber antioxidants, show strong fluorescence bands with peaks at 2·34 and 2·42 $\times$ 10^4 cm^{-1} (Figs. 9 (*a*) and 9 (*c*)). In the extracts from vulcanised rubbers the strong absorption by pine tar and other constituents masks the absorption spectra of the antioxidants, but their fluorescence spectra are

unaffected and they can be directly determined by fluorimetric analysis of the extract. Either 366 or 313-mμ light can be used for excitation. Diphenylamine (see Fig. 9 (*e*)) and carbazole (see Fig. 9 (*h*)) are examples of substances whose fluorescence emission bands are situated almost entirely in the ultra-violet region. They do not absorb appreciably at 366 mμ, and the 313-mμ mercury line had to be used for excitation. Diphenylamine is a common stabiliser for nitrocellulose propellants and, although it shows strong absorption in the ultra-violet region, this absorption is frequently masked if other additives are present. For example, in Fig. 9 (*f*) the absorption due to the diphenylamine in a propellant extract is completely masked by the presence of dinitrotoluene. Nevertheless, the fluorescence spectrum is unaffected and can be used for the quantitative determination of the diphenylamine. The curves in Fig. 9 (*g*) provide a good example of the objectionable effects produced by photo-decomposition. An attempt was made to increase the sensitivity of the method by using ultra-violet light between 250 and 300 mμ for irradiation of the diphenylamine. After a few minutes' exposure to this light, the diphenylamine solution showed the distorted fluorescence spectrum shown in Fig. 9 (*g*), from which it is apparent that an appreciable proportion of the diphenylamine had been converted to carbazole, although the proportion was not sufficient to produce a marked change in the absorption spectrum.

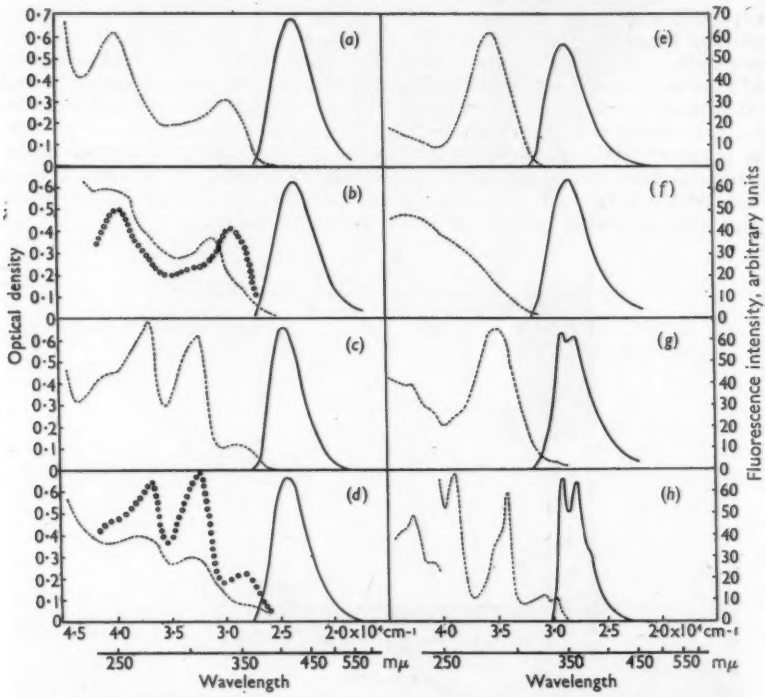

Fig. 9. Absorption and fluorescence spectra in absolute ethanol: (*a*) pure N-phenyl-1-naphthylamine; (*b*) extract of rubber containing N-phenyl-1-naphthylamine; (*c*) pure N-phenyl-2-naphthylamine; (*d*) extract of rubber containing N-phenyl-2-naphthylamine; (*e*) pure diphenylamine; (*f*) extract of a nitrocellulose propellant containing dinitrotoluene and diphenylamine; (*g*) diphenylamine after irradiation with ultra-violet light; (*h*) pure carbazole

Because the quantum yield of fluorescence is, for many compounds, constant throughout a large part of the absorption spectrum, the intensity of fluorescence of a sufficiently dilute solution is, for equal exciting intensities, proportional to the extinction coefficient. Obvious use of this can be made in the analysis of mixtures by measuring the intensity of fluorescence emission (of appropriate wavelength) with exciting light of several wavelengths. A very

simple example is the analysis of a mixture of diphenylamine and N-phenyl-2-naphthylamine in the presence of other absorbing but non-fluorescent compounds. The intensity of fluorescence emitted at $2{\cdot}42 \times 10^4$ cm^{-1} with excitation by pure 366-mμ light gives a direct measure of the N-phenyl-2-naphthylamine, while the intensity at $2{\cdot}83 \times 10^{-4}$ cm^{-1} with 313-mμ irradiation gives a direct measure of the diphenylamine. In general, the fluorescence and absorption curves for the two compounds may be sufficiently similar to make necessary the solving of simple simultaneous equations, but even then the opportunity of varying not only the exciting wavelength but also the detecting wavelength renders the composite fluorescence method more selective than methods based solely on the observation of composite absorption curves.

Measurement of fluorescence "excitation" spectra

When the fluorescence quantum yield remains substantially constant throughout the absorption spectrum, a plot of fluorescence intensity against exciting wavelength at constant intensity (the "excitation" spectrum) reproduces the absorption spectrum of the fluorescent compound. To obtain maximum sensitivity the fluorescent light is isolated by means of a broad band-pass filter centred on the peak of the emission spectrum. The method then provides a useful means of measuring absorption spectra of fluorescent compounds at concentrations much lower than those required for absorption measurements. The spectra can also be measured in the presence of non-fluorescent impurities having much higher extinction coefficients and, by suitable choice of secondary filters, the excitation spectra of the components of mixtures of fluorescent compounds with emission bands at different wavelengths can be determined directly.

Two examples of the application of excitation spectra are shown in Figs. 9 (*b*) and (*d*) (by the circles). Here, the strong absorption by other components in the extracts of the vulcanised rubbers almost completely masks the absorption due to the antioxidants present. The latter, however, show their characteristic fluorescence excitation spectra, which are unaffected by the other absorbing compounds present (provided that the solutions are sufficiently dilute to avoid an inner-filter error), and are similar to the absorption spectra of the pure antioxidants. For the purpose of identification, the excitation spectra are more specific than the emission spectra, because the latter generally correspond to transitions only from the lowest excited state, irrespective of the wavelength used for excitation.

Sensitivity

The sensitivity of the spectrofluorimeter depends not only on the sensitivity of the detector and the intensity of the exciting light, but also on the slit width and aperture of the analysing monochromator. With the narrow band-width required for the resolution of fine structure, such as that in the anthracene spectrum, the sensitivity is correspondingly low (with the small quartz double monochromator, a concentration of at least 0·1 μg of anthracene per ml is required). The slits can, of course, be opened considerably when the fluorescence band shows no fine structure. With very wide slits, the monochromator acts as a convenient infinitely variable band-pass filter. When used in this way, it avoids the difficulty associated with the choice of an efficient non-fluorescent secondary filter. For very high sensitivities the monochromator must still be replaced by a suitable secondary filter. Typical sensitivities found with the various arrangements are shown in Table I.

If quoted values of instrumental sensitivity are to be of use for comparison by other workers, they must include a statement of the dose rate of exciting light used and the value of the instrumental blank due to irrelevant fluorescence, scattered light and so on. In Table I the quoted limiting sensitivity when filters were used for isolation of the fluorescence (2×10^{-6} μg of quinine sulphate per ml) is far higher than the blank, so that with the quoted exciting intensity the high instrumental sensitivity is wasted. If, however, it is desired to use exciting light from a monochromator, *e.g.*, for the measurement of "excitation" spectra, a much lower exciting intensity will normally be available and the high sensitivity obtainable by the use of filters for isolation of the fluorescent light is then very desirable.

Choice of exciting wavelength for quantitative fluorimetry

The most generally used exciting wavelength in analytical applications is that of the 366-mμ group of mercury lines. However, it is always worth considering the possible

advantages of using one of the other strong mercury lines, *e.g.*, 254, 313, 405 or 436 mμ. Sometimes the choice of another line is essential (for example, with diphenylamine, for which the absorption at 366 mμ is negligible) but even in other cases, when adequate sensitivity can be attained with 366-mμ light, other factors may make another wavelength more desirable.

TABLE I

SENSITIVITIES OF SPECTROFLUORIMETERS

Mazda 27M3 photomultiplier tube run at 800 V (dry batteries) or, with glass-prism spectrometer, RCA 931A photomultiplier tube run at 500 V (dry batteries)

Irradiating intensity at 366 mμ corresponded to 0·2 micro-einsteins per minute

The quoted sensitivities are calculated values on the assumption that the total water blank was zero

Method of isolation of fluorescence	Slit range at 460 mμ, mμ	Fluorescence blank with water (expressed as equivalent concentration of quinine sulphate), μg per ml	Minimum detectable concentration of quinine sulphate, μg per ml
Quartz double monochromator D222/D205	5·8	not detectable	0·006
	30	0·0006	0·0002
Glass spectrometer D186	30	0·0008	0·0001
Chance OY8 glass filter with 10-mm aperture	Cut-off with 50% transmission at 468 mμ (transmits about 1/3rd of quinine fluorescence)	0·0023	2×10^{-4}

Such a situation arises when the fluorescent compound has to be determined in the presence of other compounds that absorb very strongly at 366 mμ. An example that can often occur is the determination of a non-fluorescent component by the addition of a reagent that reacts with it to form a fluorescent complex—

$$\underset{\text{(non-fluorescent)}}{X + R} \rightleftharpoons \underset{\text{(fluorescent)}}{XR}$$

The determination of traces of borate by the addition of excess of benzoin is such as example. Here the alkaline solution of benzoin alone has a comparatively high absorption at 366 mμ (see Fig. 15, p. 615). Hence it acts as an inner filter and reduces the effective irradiating intensity by an amount that varies with the concentration of benzoin used. The error introduced by the inner filter effect will, of course, depend on the geometry of the particular fluorimeter. If parallel exciting light is used and this geometry is accurately defined, a correction factor for absorption can be calculated. Even then, if the absorption is too great, the correction factor becomes large and of doubtful accuracy, and in any event the application of large correction factors requires an accurate measurement of the optical density of the solution. The geometry used with the constant-temperature cell holder (Fig. 5) is shown in Fig. 10 (*a*), together with the correction curve in Fig. 10 (*b*), which is calculated from the equation—

$$\text{Correction factor} = \frac{F_0}{F} = \frac{2{\cdot}303D\,(x_2 - x_1)}{10^{-Dx_1} - 10^{-Dx_2}},$$

where D is the optical density per cm.

The results of an investigation of the effect of the concentration of benzoin on the formation of the fluorescent borate - benzoin compound are shown in Fig. 11. The fluorescence corresponding to each concentration of benzoin was measured with both 366 and 405-mμ exciting light. The optical densities of the solutions at these two wavelengths were also measured. At 405 mμ the optical densities, even with the highest concentrations of benzoin, were small, so that the correction factors for the inner-filter effect were also small. At 366 mμ, however, the optical densities were much greater and, for the higher concentrations of benzoin, the corrections for the inner-filter effect were considerable. Curve B, Fig. 11, shows the observed fluorescence intensities at 405 mμ and curve C the same results after correction for the inner-filter effect. The latter curve represents the true concentration of

the fluorescent compound. At 366 mμ (curve A) the inner-filter effect is so great that at high concentrations of benzoin the observed fluorescence decreases with increasing benzoin concentration.

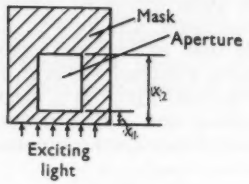

Fig. 10(*a*)

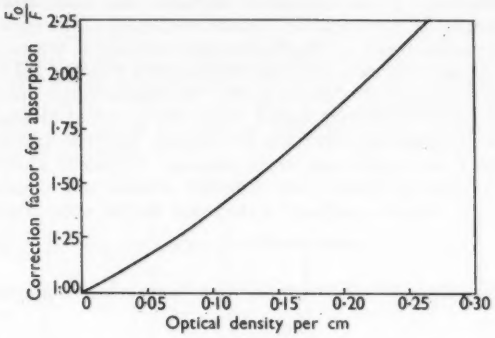

Fig. 10(*b*)

Fig. 10. Correction for absorption of exciting light: (*a*) geometry of the cuvette; (*b*) calculated correction curve for $x_1 = 0{\cdot}45$ cm and $x_2 = 2{\cdot}4$ cm

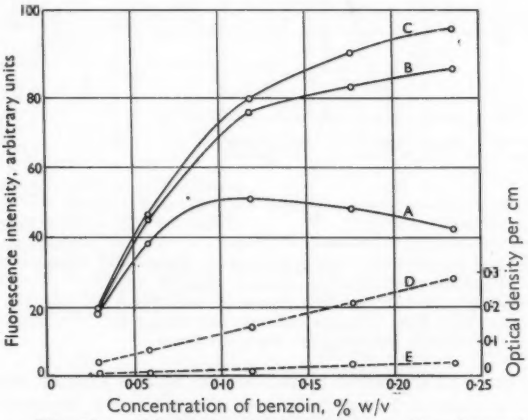

Fig. 11. Effect of optical density on the observed fluorescence, measured in a cuvette having the geometry of that in Fig. 10 (*a*), of a solution containing 0·023 μg of boron per ml in 0·005 *N* aqueous ethanolic sodium carbonate: curves A and B, observed fluorescence excited by light of wavelengths 366 mμ and 405 mμ, respectively; curve C, curve B corrected for the inner-filter effect; curves D and E, optical densities per cm at 366 mμ and 405 mμ, respectively

It is, of course, still possible to use the fluorescence excited by 366-mμ light in an empirical way for the determination of the borate concentration, and, provided that the concentration of benzoin is large compared with that of the borate (so that the optical density at 366 mμ is substantially unaffected by the concentration of borate), a linear calibration graph will be obtained. It is then essential to control the concentration of benzoin very accurately, and to exclude oxygen rigorously, to avoid changes in the optical density at 366 mμ.

Effect of oxygen

Oxygen can interfere in several ways, sometimes at quite low concentrations, and this can again be seen by reference to the borate - benzoin reaction. Two effects of oxygen are shown in Fig. 12. By using the special reaction cell, a solution of benzoin (in the trap) and

a second solution of borate in alkaline aqueous ethanol (contained in the cuvette) were both completely de-oxygenated by means of a current of purified nitrogen. The solution in the trap was then allowed to flow into the cuvette and allowed to react to give the fully developed fluoresence. If the current of nitrogen was maintained, this fluorescence intensity remained substantially constant for 2 hours (curve A). The experiment was repeated, but after reaction and measurement of the fluorescence intensity, 0·9 per cent. v/v of oxygen was injected into the nitrogen stream and the fluorescence rapidly fell to a lower value (curve B). When the oxygen supply was interrupted, the fluorescence intensity rose again, although it did not quite reach its initial value. The whole cycle of events could then be repeated again and again by periodic injection of oxygen, but the steady fluorescence intensities, both in the presence of oxygen and in its absence, decreased after each cycle (curve B). It is clear that the oxygen produces two different effects, a reversible quenching and a slow irreversible reaction, which results in a decrease in the concentration of the fluorescent compound.

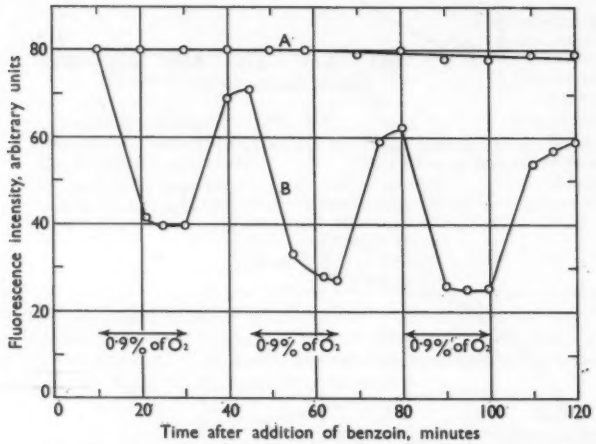

Fig. 12. Quenching of fluorescence, exited by 405-mμ light, by oxygen for the borate-benzoin compound in aqueous ethanol, the solution containing 0·018 μg of boron per ml: curve A, with pure nitrogen passing; curve B, with pure nitrogen passing, but with 0·9 per cent. v/v of oxygen periodically injected into the gas stream

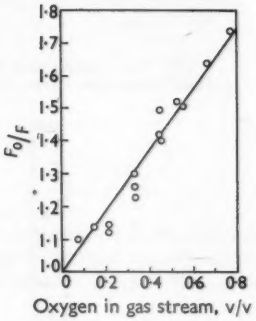

Fig. 13. Fluorescence quenching of borate-benzoin compound by oxygen

The quenching may be expected to follow the Stern - Volmer relationship—

$$F_o/F = 1 + K[O_2],$$

where K is the quenching constant. The relationship between quenching and oxygen concentration was therefore determined in a series of separate experiments in each of which the benzoin solution and the borate solution, saturated with nitrogen containing known concentrations of oxygen, were allowed to react together and the fluorescence intensities were determined, with the results shown in Fig. 13. Within experimental error they follow the Stern - Volmer law up to an oxygen concentration of about 0·8 per cent. v/v (at higher concentrations the results were erratic, partly owing to the incidence of the irreversible oxidation). Two typical values taken from this curve, together with the value obtained with air, are as follows, together the corresponding percentage errors that would be introduced—

Oxygen in gas, % v/v ..	0·1	0·8	20
F_o/F	1·09	1·74	17
Quenching error, %	8	43	94

It is clear that the concentration of oxygen must be reduced to a low value before a sensitive and consistent analytical method can be devised with this system.

By using a calculated value of the solubility of oxygen in the aqueous ethanol used, the Stern - Volmer quenching constant, calculated from the results in Fig. 13, was $0{\cdot}6 \times 10^5$ litres per mol. This is some 1000 times greater than that of, for example, anthracene in

chloroform, and indicates that if the quenching mechanism is collisional, the lifetime of the excited fluorescent state must be exceptionally long (more than 10^{-5} second). The quenching may, however, be due to molecular compound formation between the fluorescent molecule and molecular oxygen—

$$F + O_2 \rightleftharpoons FO_2.$$

If the oxygen compound were non-fluorescent, a relationship identical with the Stern - Volmer equation would be obtained, in which the quenching constant K would be the equilibrium constant for the molecular compound formation. There is in fact some evidence for compound formation, because small reversible changes in optical density of the solution at 366 mμ have been observed on addition of oxygen.

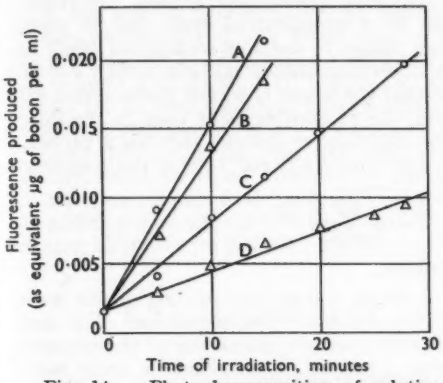

Fig. 14. Photo-decomposition of solutions containing 0·12 per cent. w/v of benzoin in 72 per cent. w/w aqueous ethanol, 0·005 N with respect to sodium carbonate, the irradiating intensity being 0·26 micro-einsteins per minute at 366 mμ: curve A, 0·45 per cent. v/v; curve B, 0·22 per cent. v/v; curve C, 0·07 per cent. v/v of oxygen injected into the gas stream; curve D, pure nitrogen

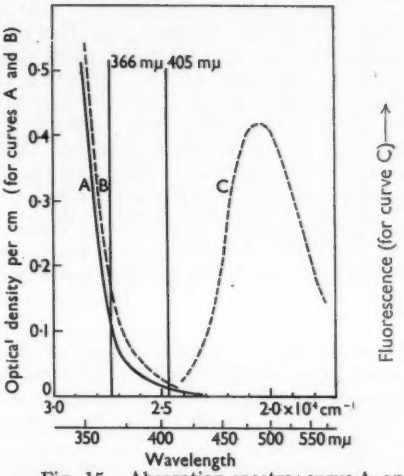

Fig. 15. Absorption spectra: curve A, an alkaline 0·12 per cent. w/v solution of benzoin; curve B, with 0·15 μg of boron added per ml. Fluorescence spectrum (curve C) of the solution with 0·15 μg of boron added per ml.

The absorption of the alkaline benzoin solution increased at wavelengths greater than 360 mμ when it was treated with oxygen or exposed to light

There are other ways in which oxygen can interfere. For example, it may irreversibly oxidise the reagent, giving an increase in optical density of the solution and hence an inner-filter error (this occurs with benzoin at 366 mμ), or giving rise to unwanted fluorescent impurities. Another remarkable effect of oxygen is seen from Fig. 14. This shows the effect of irradiating an alkaline solution of benzoin with light of wavelength 366 mμ and of intensity similar to that frequently used in fluorimeters (0·26 micro-einstein per minute). With no added oxygen a moderate photo-decomposition took place with the production of fluorescent material. The presence of a small amount of oxygen accelerated the photo-decomposition considerably.

In view of the wide variety of effects produced by the presence of oxygen (sometimes at low concentration), it is obviously necessary to investigate its possible effects with any new fluorescent system proposed for quantitative analysis. Unless the oxygen content is carefully controlled, preferably at a very low level, very irregular results may be obtained.

Photo-decomposition

Under normal working conditions the fraction of light absorbed by the fluorescent solute is small and proportional to its concentration. The fluorescence intensity is then given by the expression—

$$F = fI_0\ (2{\cdot}3\ \epsilon cd),$$

where f is the fluorescence yield, I_0 the irradiating intensity (einsteins per minute), ϵ the

decadic molar extinction coefficient, c the concentration and d the optical depth. The rate of photo-decomposition is also proportional to the light absorbed and is given by—

$$R = \phi I_0 (2{\cdot}3\ \epsilon cd) \text{ moles per minute,}$$

where ϕ is the quantum efficiency of the decomposition. In analytical work one is normally interested in the fractional rate of decomposition—

$$\frac{R}{cv} = \phi I_0 \frac{(2{\cdot}3\ \epsilon d)}{v},$$

where v is the volume of solution. This is theoretically independent of the concentration of fluorescent solute.

The fluorescence sensitivity, that is the fluorescence per unit concentration, is equal to fI_0 $(2{\cdot}3\ \epsilon d)$ and an attempt is often made to make this as high as possible by using the maximum available light intensity, particularly if a fluorescence spectrometer is used. Increase in exciting intensity will at the same time result in a proportional error due to photo-decomposition, and it is worth substituting actual values to see how serious the effect can be in an unfavourable case. Assume a strongly absorbing fluorescent solute with $\epsilon = 10^4$, and with $d = 4$ cm and $v = 15$ ml. Assume also that the solute is highly photo-active with quantum efficiency $\phi = 0{\cdot}5$. The maximum dose rate of exciting light used in the fluorimeters described above is approximately 0·3 micro-einsteins per minute, and this is probably typical of many fluorimeters. With this dose rate the fractional rate of decomposition (R/cv) would be—

$$0{\cdot}5 \times (0{\cdot}3 \times 10^{-6}) \times \frac{(2{\cdot}3 \times 10^4 \times 4)}{0{\cdot}015}$$

$$= 0{\cdot}92 \text{ per minute.}$$

Assuming that it takes 6 seconds of irradiation to obtain a duplicate reading at one wavelength, this would introduce an error of 9 per cent. by photo-decomposition. For some purposes this might be acceptable, but if the fluorescence of the solution has to be repeatedly measured, *e.g.*, to obtain its fluorescence emission spectrum, a considerable error would be introduced.

The photo-decomposition of other constituents of the solution can, in unfavourable instances, introduce even greater errors. An example of particular interest in trace analysis is the addition of a reagent, R, to combine with the substance to be determined to give a fluorescent product. R must normally be added in excess, and it may well decompose photo-chemically to give fluorescent products. Since this rate of decomposition will be independent of the concentration of fluorescent solute being measured, the percentage error introduced will increase as the concentration of the latter decreases. Again it is worth calculating the order of magnitude of the effect to be expected. In the following, the "dashed" symbols refer to the fluorescent compound produced by the photo-decomposition of R. The rate of formation of this compound is given by $\frac{\phi' I_0 x}{v}$ moles per litre per minute (where x is the fraction of light absorbed by R) and the corresponding rate of production of fluorescence by—

$$\Delta F' = \frac{\phi' I_0 x}{v} f' I_0 (2{\cdot}3\ \epsilon' d).$$

Expressing this as a fraction of the fluorescence due to the substance to be determined gives the error introduced per minute of irradiation—

$$\frac{\Delta F'}{F} = \frac{\phi' I_0 x}{v} \cdot \frac{f' I_0 (2{\cdot}3\ \epsilon' d)}{f I_0 (2{\cdot}3\ \epsilon dc)} = \frac{\phi' I_0 x}{cv} \cdot \frac{f' \epsilon'}{f \epsilon}.$$

An unfavourable case might have $f'\epsilon' = f\epsilon$, $\phi = 1$, $x = 0{\cdot}1$ and $c = 10^{-7}\ M$. Assuming, as before, $I_0 = 0{\cdot}3 \times 10^{-6}$ and $v = 0{\cdot}015$ then—

$$\frac{\Delta F'}{F} = 20.$$

Under these conditions an exposure of $\frac{1}{100}$th minute would produce an error of 20 per cent. Even taking very much less unfavourable conditions, it is easy to see that considerable errors can still arise, particularly if a number of readings has to be taken. This situation is in fact found in the fluorimetric determination of boron. It proved difficult to determine

the true fluorescence spectrum of the compound at low concentrations of boron with narrow spectrometer slits, owing to the rapid photo-decomposition of the excess of benzoin with irradiating intensities (366 mμ) sufficient to give adequate response with the photo-multiplier tube with narrow slits. Fortunately the fluorescence band is broad in this case and wider slits and lower irradiating intensity could be used for quantitative measurements.

The absorption of the alkaline benzoin solution shown in curve A, Fig. 15, is considerably less at 405 mμ than at 366 mμ and the rate of photo-decomposition at this wavelength is correspondingly lower. Unfortunately, the absorption of the boron compound, shown in curve B, Fig. 15, is also less at 405 mμ, and it could not be predicted whether any net advantage would be gained by using this wavelength. Some measurements were therefore made of the relative fluorescence sensitivities and the relative rates of photo-decomposition at the two wavelengths. From column 4 of Table II it is seen that the fluorescence sensitivity at 405 mμ is only 15 per cent. of that at 366 mμ for equal exciting intensities. On the other hand, the rate of decomposition at 405 mμ (in terms of the fluorescence as measured by 405-mμ irradiation) is only 9 per cent. of that at 366 mμ (in terms of the fluorescence as measured by 366-mμ irradiation) when compared on the basis of equal irradiating intensities (see column 4 of Table III). Hence, if the intensity at 405 mμ is increased (by a factor of 100/15) to give the same fluorescence sensitivity as that obtained at 366 mμ, the rate of photo-decomposition is still less than that observed with 366-mμ irradiation (see column 5 of Table III). If, in addition, the inner-filter effect is taken into account, the rate of photo-decomposition for equal fluorescence sensitivities is some 2½ times less at 405 mμ than at 366 mμ (see column 6 of Table III). It should be remarked that 405-mμ irradiation has the additional advantage of a much smaller inner-filter error. It has the disadvantage that existing filters for its isolation are not so convenient in use.

TABLE II

RELATIVE FLUORESCENCE INTENSITIES AT DIFFERENT WAVELENGTHS

For the borate - benzoin compound, with 0·012 μg of boron per ml

Wavelength, mμ	Intensity of exciting light, micro-einsteins per minute	Fluorescence intensity corrected for inner-filter effect	Relative fluorescence for equal exciting intensities
366	0·00306	100	1·00
405	0·00148	7·2	0·15
436	0·00294	0·7	0·007

TABLE III

RELATIVE RATES OF PHOTO-DECOMPOSITION

Alkaline aqueous ethanolic benzoin solution, containing 0·118 per cent. w/v of benzoin; saturated with nitrogen containing 0·3 per cent. of oxygen

Wavelength, mμ	Intensity of irradiating light, micro-einsteins per minute	Rate of decomposition expressed as equivalent concentration of boron, μg per ml per minute	Relative rate of decomposition at the same intensity	Relative rates at intensities which give equal fluorescence sensitivity	
				Without inner-filter effect	With inner-filter effect
366	0·084	0·00073	1·00	1·0	1·5
405	0·061	0·00005	0·09	0·6	0·6

MEASUREMENT OF EXCITING INTENSITIES

The dose rate of ultra-violet light received by the contents of the fluorimeter cuvette can be very simply measured by means of the ferrioxalate actinometer,[3,4] and such measurements are of considerable value in fluorimetric investigations. For example, the sensitivity of a particular fluorimeter will depend directly on the intensity of exciting light used, and quoted values of instrumental sensitivity will be of most use to other workers if this intensity is also quoted. The values of the exciting intensities are also required if photochemical effects or fluorescence sensitivities at different wavelengths are being compared. Chemical actinometry is also a convenient method of comparing the relative intensities obtainable from different ultra-violet lamps or from different wavelengths with the same lamp.

Typical ultra-violet dose rates obtainable at various wavelengths from two types of mercury-vapour lamp in common use are shown in Table IV. These were incidentally determined for another purpose,[4] but they are reproduced here in the belief that they will be useful to those concerned with fluorimetric measurements. The following points are of particular interest. First, the intensities obtainable from a small double monochromator (D222/D205) are 50 to 100 times less than those obtainable by using filters for the isolation of the lines. If a very wide aperture monochromator (D96) is used, comparable intensities

TABLE IV

TYPICAL DOSE RATES OBTAINED FROM MERCURY-VAPOUR LAMPS (AS MEASURED BY MEANS OF A FERRIOXALATE ACTINOMETER IN A 15-ml CELL)

Wavelength, mμ	Dose rate with 125-watt lamp type MB/D with filters, micro-einsteins per minute	Dose rate with 125-watt lamp type MB/D with D222/D205 double monochromator, micro-einsteins per minute	Dose rate with a 1-kW compact-source lamp with D96 wide-aperture monochromator, micro-einsteins per minute
577/9	0·5	0·021	—
546	1·0	0·027	—
436	2·1	0·020	3·7
405	0·5	0·009	2·2
366	2·0	0·020	2·7
334	—	0·002	1·2
313	0·6	0·008	1·5
302	—	0·004	1·3†
265	—	0·001	—
254	0·1*	—	—

* Low-pressure lamp. † Including 297-mμ light.

to those isolated by filters can be obtained, and with higher spectral purity. The other point of particular importance concerns the relative intensity of the 334-mμ mercury line. With the high-pressure lamp (type MB/D) this line is comparatively weak (about 5 to 10 per cent. of the 366-mμ line) and is usually neglected when filters are being selected for the isolation of either the 366-mμ or the 313-mμ line. With the compact-source type of lamp (1 kW) the intensity of the 334-mμ line is relatively much stronger (40 per cent. of the 366-mμ line) and consequently, when this type of lamp is used in conjunction with the usual filters, the 366-mμ or 313-mμ light obtained is very impure and will introduce considerable errors into fluorimetric calculations based on the assumption of monochromatic exciting light. Hence typical transmission values for Chance OX1 glass (Spekker H556 filter) are 46 per cent. at 334 mμ and 61 per cent. at 366 mμ, and even ordinary window glass transmits about 35 per cent. at 334 mμ. With 1 mm of ON12 didymium glass in conjunction with OX1 glass, 366-mμ light can be isolated from 334-mμ light (transmission of didymium glass is less than 0·5 per cent. at 334 mμ and 52 per cent. at 366 mμ). A Spekker H503 filter can also be used (33 per cent. at 366 mμ). The relative intensities of the 313, 334 and 366-mμ lines from 250-watt compact-source lamps were found to vary, but in general the intensity at 334 mμ was only 10 to 15 per cent. of that at 366 mμ, although it was still roughly comparable with that at 313 mμ.

Although the high intensity at 334 mμ from a 1-kW compact-source lamp is objectionable when the 313 mμ or 366-mμ lines are required pure, it forms a useful source of high-intensity monochromatic light at a wavelength in the wide gap between 313 and 366 mμ.

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The Determination of Antimony in Cast Iron

By R. C. ROONEY

A method is described for the determination of antimony in cast iron. The sample is dissolved in nitric acid under controlled conditions. The residue, consisting of graphite and silica, is filtered off, and antimony is determined separately in the residue and in the filtrate.

The residue is ignited and silica is then removed by volatilisation as the fluoride. It is then taken into solution by fusion with potassium hydrogen sulphate, the acidity is adjusted to 4 *N* with sulphuric acid, the coloured iodide is formed and the determination is completed spectrophotometrically. The filtrate is treated with potassium permanganate solution to oxidise combined carbon, the excess of permanganate being reduced with hydrogen peroxide. The pH is adjusted to 1 and antimony is co-precipitated on manganese dioxide by addition of permanganate. The precipitate is redissolved and tested for iron. If the iron content is less than 0·05 g, the acidity is adjusted and the determination of antimony completed as for the residue. If more than 0·05 g of iron is present, a further precipitation is carried out.

The correct conditions for separation were examined, as well as the conditions for the spectrophotometric determination as iodide. Interferences were studied and it was shown that the interference from bismuth was negligible under the given conditions.

ANTIMONY is usually present in cast iron as a trace constituent, normally less than 0·1 per cent. and frequently less than 0·01 per cent. Most of the previously published methods for its determination have made use of a separation of the antimony as sulphide by means of hydrogen sulphide, with the final determination made by oxidimetric titration.[1,2] These procedures were investigated with a view to their applicability to the determination of microgram amounts of antimony, but were found to be unsatisfactory. It was also considered desirable to replace the separation with hydrogen sulphide, which is both tedious and unpleasant.

EXPERIMENTAL

The method of Westwood and Mayer[2] was investigated thoroughly, and found to be unsatisfactory in several respects. Although quite good agreement can be obtained between duplicate determinations performed simultaneously, this agreement is not maintained with a new batch of determinations. Recovery of added antimony is very poor, and leads to suspicion of either the sulphide separation or the final titration with chloramine T or both. This titration is unsatisfactory; the end-point is sluggish and uncertain, and marked fading takes place. It is very difficult to wash the sulphide precipitate free from iron, which leads to erroneous figures. The precipitate is also difficult to filter off, as there is a considerable amount of colloidal material present.

The method recommended for the elimination of arsenic before titration, by boiling with concentrated hydrochloric acid in an open 150-ml squat beaker, is very susceptible to mechanical loss.

Another method that has been recommended for the separation is volatilisation of the antimony as stibine.[3,4] A considerable amount of work was done on this procedure, as it is nearly specific for antimony, and would provide a very rapid method of separation. Unfortunately, this procedure was also found to be unsatisfactory, as the instability of the hydride is such that it is virtually impossible to obtain a yield of of 100 per cent. from antimony solutions containing iron. It was also found that the proportion of antimony obtained as stibine varied with several factors, including the batch of zinc used as reducing agent, the composition of the sample and the particular apparatus used. For example, two apparently identical 25-ml flasks were used, with absorption tubes fitted directly into the necks. One of these gave a constant yield of 65 to 70 per cent., whereas the other gave yields that varied between 11 and 46 per cent.

Deposition of antimony on strips of metallic copper has been used as a method of separation, but results obtained by this method showed a tendency to be low. This may be explained by the recent work of Babko and Shtokalo,[5] who have shown that washing of the copper foil results in loss of antimony. These workers also showed, by using antimony-124 as a tracer, that the co-precipitation of antimony on manganese dioxide formed from potassium

permanganate and manganous salts[6,7] is complete in the pH range 1 to 7. This method of separation was used for the subsequent work.

Of the available methods for the final determination of antimony, it was decided to discard the volumetric procedure in favour of a spectrophotometric one. It was considered that the oxidation titration was subject to too many interferences, especially from iron, and that semi-micro titrations were unsuitable for use in routine laboratories.

Of the various colorimetric reagents available, potassium iodide and methylfluorone (9-methyl-2:3:7-trihydroxy-6-fluorone) were investigated. Rhodamine B was not examined, since it is too subject to interference. Methylfluorone was found to suffer from the same disadvantage as rhodamine B, and potassium iodide was therefore chosen, since it is readily available and gives stable calibration curves.

The conditions for the determination of antimony with potassium iodide were first investigated, with the conditions recommended by Sandell[8] as a starting point. The time for colour development was found to be 5 minutes, the colour being stable for at least 30 minutes, and the wavelength of maximum absorption was found to be 425 mμ. These findings agree with the published data. The colour intensity was found to be practically unchanged over the range 3 to 5 *N* in sulphuric acid, but, at acid concentrations much above this, decomposition of the potassium iodide occurred.

The variation of colour intensity with iodide concentration was studied. It was found that there was little variation and that the sensitivity was satisfactory in the range 15 to 25 per cent. of potassium iodide.

With these conditions, a calibration curve was plotted over the range 0·05 to 0·50 mg of antimony, with a volume of 50 ml and a 2-cm cell. This calibration curve was a straight line passing through the origin and having a slope such that 0·5 mg of antimony gave an optical density of 0·8. A second curve covering the range 0·5 to 2·5 mg was also plotted, a 0·5-cm cell being used.

The conditions required for the co-precipitation of antimony with manganese dioxide were next studied.

In order to obtain a pH in the range specified by Babko and Shtokalo, the minimum amount of acid must be used to dissolve the sample, but care must be taken that the pH does not rise sufficiently for hydrolysis of the ferric iron to occur. It was found that the optimum acid concentration for dissolution of a 5-g sample was 120 ml of diluted nitric acid (1 + 4). This gives a pH at the precipitation stage of 1 or slightly over. With lower acid concentrations, sufficient acid can be lost during the initial stages to lead to deposition of basic ferric salts with the manganese dioxide.

Additions of standard antimony solution were now made to 5-g portions of pure iron, and the recovery of the method was checked. The results were as follows—

Antimony added, % ..	Nil	0·0020	0·0040	0·0060	0·0080	0·010
Antimony found, % ..	0·0013	0·0033	0·0046	0·0074	0·0089	0·011
Recovery, %	—	100	75	100·7	95	100

The method was now used for the analysis of a group of irons known to contain antimony. It was found that a large and variable proportion of the antimony was retained on the copious graphite and silica residue. A number of methods was tried in order to achieve complete solution of all of the antimony, but they were unsuccessful. Accordingly, the residues were processed separately. The results on these irons are shown in Table I.

TABLE I

DETERMINATION OF ANTIMONY IN RESIDUE AND FILTRATE

Sample No.	Antimony found in residue, %	Antimony found in filtrate, %	Total antimony found, %
1	0·0009	0·0011	0·0020
	0·0011	0·0010	0·0021
	0·0015	0·0010	0·0025
2	0·023	0·011	0·034
	0·022	0·014	0·036
	0·034	0·0043	0·038
3	0·059	0·026	0·085
	0·061	0·025	0·086
	0·035	0·048	0·083

The standard deviation was determined for sample No. 1 of this series. The mean result of eight determinations was 0·0024 per cent. of antimony; the standard deviation was 0·0004 per cent.

INTERFERENCES—

The major sources of interference in the iodide colorimetric procedure for antimony are: (*a*) ions that are strongly coloured, (*b*) ions that yield an insoluble iodide, (*c*) oxidising agents and (*d*) ions that give a colour with potassium iodide. The manganese dioxide precipitation effectively separates antimony from coloured ions such as chromium and nickel, and from ions yielding insoluble iodides such as copper and lead. If small amounts of these ions are carried down, the large excess of iodide present is sufficient to redissolve the precipitate, with little or no increase in absorption.

The major sources of interference likely are ferric iron, which will undoubtedly be present in small amounts, and other coloured iodides.

It was found that up to 0·10 g of iron could be tolerated in the presence of the equivalent of 0·004 per cent. of antimony in a 5-g sample. With this amount of iron present the optical density corresponded to 0·0041 per cent. of antimony. Large amounts of iodine are liberated, and 10 to 15 minutes are required for the hypophosphite to reduce it again, but no further effect was noticed.

The only other ion reported to interfere that is at all likely to be present in cast iron is bismuth, which has also been separated on manganese dioxide.[9,10,11] According to McChesney,[12] this interference can be overcome since bismuth will develop its colour at a concentration of 1 per cent. of potassium iodide, whereas antimony will not. The bismuth colour can be developed and measured, and the iodide concentration is then increased and the antimony plus bismuth colour is developed and measured. Antimony is found by difference.

Calibration curves for bismuth were constructed, and additions of equal amounts of both antimony and bismuth were made to a series of 5-g samples of pure iron. The results were as follows—

Antimony and bismuth added, % ..	0·0020	0·0040	0·020	0·030
Bismuth found, %	0·0005	0·0009	0·0036	0·0059
Recovery of bismuth, %	25	25	18	20
Antimony found, %	0·0022	0·0042	0·019	0·029

From these results it can be seen that the recovery of bismuth is very poor and is fairly consistent at about 20 per cent. In addition to this, the wavelength of maximum absorption for bismuth is 460 mμ, which further decreases its interference. The ratio of the absorption of bismuth at 425 mμ to that of antimony at 425 mμ is such that 0·010 per cent. of bismuth gives an apparent antimony content of 0·004 per cent. If only a 20 per cent. recovery of bismuth is effected, then an iron containing 0·010 per cent. of bismuth would give antimony figures less than 0·001 per cent. higher than the true value. If large amounts of bismuth are present with small amounts of antimony, correction must be made by the method of McChesney.

METHOD

REAGENTS—

Nitric acid, concentrated.

Nitric acid, diluted (1 + 4)—Dilute 200 ml of nitric acid, sp.gr. 1·42, to 1 litre with water.

Sulphuric acid, diluted (1 + 4)—Dilute 200 ml of sulphuric acid, sp.gr. 1·84, with 800 ml of water; cool and dilute to 1 litre.

Sulphuric acid, 20 N—Dilute 560 ml of sulphuric acid, sp.gr. 1·84, with 440 ml of water; cool and dilute to 1 litre.

Hydrofluoric acid, 40 *per cent.*

Hydrochloric acid, concentrated.

Potassium hydrogen sulphate.

Iodide - hypophosphite solution—Dissolve 500 g of potassium iodide and 100 g of sodium hypophosphite in water and dilute to 1 litre.

Sodium thiosulphate solution—Dissolve 10 g of sodium thiosulphate and 1 g of anhydrous sodium carbonate in water and dilute to 1 litre.

Potassium permanganate solution, 25 g per litre.

Hydrogen peroxide, 20-volume.

Ammonium nitrate solution, 20 g per litre.

Standard antimony solution—Dissolve 6·6875 g of pure dried antimony potassium tartrate in water and dilute to 500 ml.

1 ml ≡ 0·005 g of Sb (≡ 0·10 per cent. on 5 g).

PROCEDURE—

For antimony contents of less than 0·05 per cent., weigh 5 g of sample and dissolve it in 120 ml of diluted nitric acid (1 + 4). For antimony contents of 0·05 to 0·25 per cent., weigh 1 g of sample and dissolve it in 30 ml of diluted nitric acid (1 + 4), and then add 50 ml of water and filter off the graphite and silica.

Weigh the sample into a 400-ml squat beaker and cautiously add the acid. Keep the beaker well away from all sources of heat until the sample has dissolved, in order to prevent deposition of basic iron salts on the walls of the beaker. When dissolved, heat to boiling and filter through a paper-pulp pad into a 400-ml conical beaker, washing well with hot water. Transfer the residue completely to the pad with the aid of a rubber-tipped glass rod. Transfer the liquid in the column to the filtrate by gently lifting one side of the pad with a spatula.

Treatment of the residue—Transfer the pad to a porcelain or silica crucible, and transfer any residue adhering to the walls of the funnel by wiping with filter-paper. Ignite at approximately 600° C until all the paper and graphite are burnt away. Cool, transfer to a platinum crucible and add 10 drops of diluted sulphuric acid (1 + 4) and 2 to 3 ml of hydrofluoric acid. Evaporate to dryness, using a radiation bath, but do not ignite the residue. Fuse it with 1 to 2 g of potassium hydrogen sulphate, and then transfer the melt to a 150-ml squat beaker by leaching with water and add 10 ml of 20 *N* sulphuric acid. Evaporate just to fumes. Cool, rinse round the beaker with 3 to 5 ml of water and warm until solution is complete. Transfer the solution to a 50-ml calibrated flask, keeping the volume below 30 ml. Cool, and add 20 ml of iodide - hypophosphite reagent. Set aside for 5 minutes and then add 1 or 2 drops of starch indicator. If the blue starch - iodine complex appears, add sodium thiosulphate solution dropwise until the colour becomes a clear yellow. Dilute to 50 ml, shake and read the optical density immediately at 425 mμ, using a 2-cm or 0·5-cm cell, as required, and setting the instrument on a reagent blank.

Treatment of the filtrate—Add 10 ml of potassium permanganate solution and boil for 3 to 5 minutes. Cool slightly and add 20-volume hydrogen peroxide until all the manganese dioxide has dissolved. Boil the solution for 5 minutes to destroy excess of peroxide and adjust the volume to not less than 200 ml. Heat the solution to boiling and add 1 ml of potassium permanganate solution from a pipette; then boil it gently for 15 minutes, and allow the precipitate to settle.

With irons of very high combined carbon content, the 1 ml of potassium permanganate solution may not lead to precipitation of manganese dioxide. If no precipitation occurs after 1 to 2 minutes' boiling, add potassium permanganate solution drop by drop until the solution is strongly coloured. Boil for 3 to 5 minutes, clear the solution by adding 20-volume hydrogen peroxide and remove excess of peroxide by boiling. Adjust the volume, and add 1 ml of potassium permanganate solution, boil for 15 minutes and allow the precipitate to settle.

Collect the precipitate on a Whatman No. 541 filter-paper and wash it well with hot 2 per cent. ammonium nitrate. Open out the paper, and wash the precipitate back into the 400-ml conical beaker with hot water; put drops of concentrated hydrochloric acid and 20-volume hydrogen peroxide on the paper to dissolve the manganese dioxide adhering to it, and finally wash the paper thoroughly with hot water. Add to the solution 1 ml of 20 *N* sulphuric acid and 10 ml of concentrated nitric acid, and evaporate just to fumes.

Add 10 ml of water and warm until solution is complete. Check the iron content of the solution as follows—

Use a capillary pipette to extract approximately 1 μl from the solution, and put it on a Whatman No. 1 filter-paper. Also on the paper put 1 μl of a standard iron solution (0·5 g of

iron per 100 ml) from the same pipette. Hold the paper over the mouth of a bottle of ammonia solution, sp.gr. 0·880, and see if the yellow-brown colour of the test spot is less dense than that of the standard. If the spots are numbered in pencil, up to a dozen can be accommodated on a 11-cm filter-paper.

If the iron content is less than 0·05 *g*, add 9 ml of 20 *N* sulphuric acid and evaporate just to fumes; transfer to a 50-ml calibrated flask and complete the determination as described for the residue.

If the iron content is greater than 0·05 *g*, add 5 ml of potassium permanganate solution, 20-volume hydrogen peroxide to decolourise it, and again evaporate just to fumes. Dilute with 200 ml of water, add 5 g of ammonium nitrate, and heat to boiling. Add 1 ml of potassium permanganate solution, boil gently for 15 minutes, allow the precipitate to settle and collect it on a Whatman No. 540 filter-paper. (As this precipitate does not coagulate as readily as the first, owing to the conditions of precipitation, a Whatman No. 540 filter-paper is used.) Wash the precipitate, open the paper out, and return the precipitate to the beaker, washing the paper as described above. Add 10 ml of 20 *N* sulphuric acid and 10 ml of concentrated nitric acid, and evaporate to fumes. Complete the determination as described for the residue. If a precipitate of silica is observed in the final solution, owing to co-precipitation on the manganese dioxide, develop the colour, but filter the solution into the cell.

Calculate the antimony contents of residue and filtrate from a previously prepared calibration graph, and add the values together to give the total antimony content.

PREPARATION OF CALIBRATION GRAPH—

Dilute the standard antimony solution 10-fold, and measure 1, 2, 3, 4 and 5-ml aliquots (equivalent to 0·01 to 0·05 per cent. of antimony) into 50-ml calibrated flasks and to each add 10 ml of 20 *N* sulphuric acid, 10 to 15 ml of water and 20 ml of iodide - hypophosphite reagent and then dilute to 50 ml with water. Read the optical densities in a 0·5-cm cell, and plot a curve.

Dilute the standard antimony solution 100-fold, and measure 1, 2, 4, 6, 8 and 10-ml aliquots (equivalent to 0·001 to 0·010 per cent. of antimony) into 50-ml calibrated flasks. Develop the colour as described above and read the optical density in a 2-cm cell. Plot a second curve.

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The Determination of Tin in Zirconium and its Alloys

By D. F. WOOD and R. T. CLARK

A volumetric method is described for the determination of tin in zirconium and its alloys. The procedure is based on the direct reduction of tin with aluminium in the presence of titanous ions and subsequent oxidation of tin with a standard solution of potassium iodate.

The method is accurate over the range 0·01 to 20 per cent. of tin, is rapid and can be extended to the examination of samples containing larger amounts of this metal. No significant error is introduced by the presence of iron or nickel up to at least 20 per cent., tungsten up to 10 per cent. and copper, molybdenum or vanadium up to 5 per cent. Below 2 per cent. of tin, up to 0·5 per cent. of chromium has no effect, but, in the determination of larger amounts of tin, chromium can only be tolerated up to about 0·25 per cent. Modifications to the direct procedure are described whereby interference from larger amounts of chromium can be overcome. The standard deviation is 0·015 at the 2 per cent. of tin level.

The production for nuclear reactors of zirconium alloys containing tin, iron, chromium, nickel and so on has necessitated the provision of analytical procedures for these constituents. This paper covers the development of a procedure for the determination of tin, particularly in Zircalloy 2 and similar alloys.

A volumetric method, based on the reduction of tin to the bivalent state and subsequent oxidation with a standard solution of iodine or potassium iodate, is used extensively for the determination of tin in other materials. Many variants of this method exist, differing especially in the reducing agent used; among those recommended are lead, iron and nickel,[1] aluminium[2,3] and sodium hypophosphite.[4,5] In some instances, a catalyst, *e.g.*, antimony trichloride[3] or mercuric cyanide,[5] is also added to assist in the complete reduction of the tin. Further, the hypophosphite reduction method, introduced by Evans,[4] has been successfully applied, in our laboratories, to the routine analysis of copper and titanium alloys.

Experimental

The zirconium used for experimental purposes was tin-free. The standard tin solution was prepared by dissolving 0·5 g of pure tin (99·995 per cent.) in 250 ml of concentrated hydrochloric acid and diluting the solution with distilled water to 500 ml in a calibrated flask, so that—

$$1 \text{ ml} \equiv 1{\cdot}0 \text{ mg of tin.}$$

For use in experiments that involved the determination of less than 0·1 per cent. of tin, 25 ml of this solution were diluted with diluted hydrochloric acid (1 + 1) to 250 ml in a calibrated flask, so that—

$$1 \text{ ml} \equiv 0{\cdot}1 \text{ mg of tin.}$$

Reduction with sodium hypophosphite—

After the satisfactory application of the hypophosphite method to the determination of tin in copper and titanium alloys, experiments were made in order to assess its application to solutions containing relatively large amounts of zirconium. These were prepared in platinum dishes by dissolving 0·2-g samples of zirconium in a hydrofluoric - sulphuric acid mixture, followed by evaporation to fumes of sulphur trioxide. Solutions prepared in this way were transferred to conical flasks, the acidities were adjusted to (1 + 1) with respect to hydrochloric acid and known volumes of a standard tin solution, equivalent to 12 per cent. of tin, were added. The flasks were fitted with delivery tubes leading into a sodium bicarbonate solution and reduction was then effected by boiling gently for 15 minutes with sodium hypophosphite in the presence of mercuric chloride and titanous chloride. After cooling, with the delivery tubes still in position, potassium iodide was added and the solutions were titrated with a standard potassium iodate solution.

During the reduction, a small amount of zirconium phosphate was precipitated, and on titration with potassium iodate solution, a brown flocculent precipitate was formed. Qualitative tests established that this contained zirconium and iodine, and additional tests clearly

indicated that the presence of hypophosphite had led to its formation. In addition, the results for tin were both high and erratic, and these were associated with the formation of the precipitate. Attempts to prevent precipitation by forming complexes of zirconium with tartaric acid, ethylenediaminetetra-acetic acid or fluoroboric acid were unsuccessful; consequently alternative methods of reduction were investigated. No precipitation occurred when lead, iron or aluminium was used, the reduction with aluminium being the most rapid; this metal was therefore chosen for further investigation.

REDUCTION WITH ALUMINIUM—

Reduction of tin with aluminium in the presence of antimony trichloride is recommended in the analysis of steels[3] and this procedure was used as a basis for investigation.

Preliminary results of tests made in the presence of zirconium were low and erratic, and this was attributed to oxidation of the stannous chloride, either by incomplete exclusion of air from the solution during titration, or by oxygen dissolved in the potassium iodate solution. It has been reported[2] that interference from these effects can be eliminated by the addition of titanous ions, and the effect of titanous chloride was therefore investigated.

Effect of titanous chloride—Solutions containing 0·2 g of zirconium and the equivalent of 5 per cent. of tin were prepared in the same way as those used in the earlier hypophosphite reduction experiments. Various amounts of titanous chloride solution, containing the equivalent of 0·01 to 0·2 g of titanium, were added and the concentration of acid was adjusted to (1 + 1) with respect to hydrochloric acid. Reduction was effected by boiling gently for 15 minutes with aluminium turnings; antimony trichloride was not added, since it appeared to have no beneficial effect in the previous experiments. Normal precautions to exclude air during and after reduction were observed. After the solutions had been cooled, potassium iodide was added and the tin was determined by direct titration with a standard potassium iodate solution. Recovery of tin increased with increasing amounts of titanium up to 0·075 g and complete recovery was obtained in the presence of between 0·075 and 0·2 g of titanium. The effect of titanium on the determination of tin in solutions containing 0·2 g of zirconium and the equivalent of 5 per cent. of tin is shown by the following results—

Titanium present, g ..	0·010	0·025	0·050	0·075	0·10	0·15	0·20
Tin found, %	4·78	4·94	4·96	5·00	5·03	5·00	5·00

To prevent interference by dissolved oxygen in the potassium iodate solution, 15 ml of a titanous chloride solution, containing the equivalent of 0·15 g of titanium, were added to the solutions used in subsequent experiments.

Use of fluoroboric acid—Zirconium will not readily dissolve in sulphuric or hydrochloric acids and solution in hydrofluoric acid necessitates the use of platinum apparatus. Further, removal of hydrofluoric acid by evaporation with sulphuric acid is essential in order to prevent attack on the glassware subsequently used. Fluoroboric acid readily dissolves zirconium and, provided the acid is sufficiently dilute, glass apparatus can be used, and evaporation to fumes with sulphuric acid is not necessary. Tests were therefore made to determine the effect of this acid.

Samples of zirconium (about 0·2 g) were dissolved in a mixture of 50 ml of concentrated hydrochloric acid and 5 ml of fluoroboric acid contained in conical flasks. The equivalent of 5, 12·5 and 20 per cent. of tin was added as a standard tin solution, the acid concentration of the solutions was adjusted to (1 + 1) with respect to hydrochloric acid and the tin was determined by the proposed aluminium reduction procedure.

Immediately after addition of the potassium iodide, a precipitate of potassium fluoroborate was formed. Although the end-point of the titration was less clearly defined in the presence of this precipitate, recovery of tin was satisfactory. The effect of the presence in the solution of 5 ml of fluoroboric acid on the determination of tin is shown by the following results—

Zirconium present, g	0·19	0·175	0·15
Tin added, %	5·00	12·50	20·00
Tin found, %	5·00	12·51	19·96

To establish the reliability of the procedure in the presence of fluoroboric acid, further tests were made with hydrochloric - fluoroboric acid solutions containing nominal amounts of zirconium and the equivalent of 0·5 to 20 per cent. of tin. In these tests, sodium iodide

was used in place of potassium iodide because of the more favourable solubility of sodium fluoroborate over the corresponding potassium salt. Under these conditions, precipitation did not occur and detection of the end-point presented no difficulty. Moreover, owing to the formation of a green complex of titanium with fluoroboric acid, the colour change at the end-point (green to blue) was an improvement on that obtained in the absence of fluoroboric acid (blue to dark blue). The results were satisfactory (see Table I) and fluoroboric acid and sodium iodide were used in subsequent tests.

Table I

Determination of tin in solutions containing fluoroboric acid and sodium iodide

Zirconium present, g	Tin added, %	Tin found, %
0·20	0·50	0·50
0·20	1·00	1·01
0·20	2·00	2·03
0·19	5·00	4·97
0·18	10·00	9·98
0·16	20·00	19·96

Effect of other metals—To assess the application of this procedure to Zircalloy 2, which is a zirconium-base alloy containing approximately 1·5 per cent. of tin, 0·12 per cent. of iron, 0·1 per cent. of chromium and 0·05 per cent. of nickel, replicate determinations of tin were made on solutions containing 0·2 g of zirconium, 2·00 per cent. of tin and amounts of these metals in proportion to the amount present in the alloy. The results were 2·01, 2·02, 2·01, 2·00 and 1·98 per cent. of tin, which gave a standard deviation of 0·015.

Table II

Effect of other metals on the determination of tin

Metal	Zirconium present, g	Metal added, %	Tin added, %	Tin found, %	Zirconium present, g	Metal added, %	Tin added, %	Tin found, %
Iron	0·19	5	2·00	2·02	0·15	5	20·00	20·06
	0·18	10	2·00	2·04	0·14	10	20·00	19·96
	0·16	20	2·00	2·01	0·12	20	20·00	20·00
Nickel	0·19	5	2·00	2·04	0·15	5	20·00	19·96
	0·18	10	2·00	2·01	0·14	10	20·00	20·00
	0·16	20	2·00	1·99	0·12	20	20·00	20·03
Tungsten	0·19	5	2·00	2·04	0·15	5	20·00	19·96
	0·18	10	2·00	2·01	0·14	10	20·00	19·91
	0·16	20	2·00	—*	0·12	20	20·00	—*
Molybdenum	0·19	5	2·00	2·04	0·15	5	20·00	20·06
	0·18	10	2·00	2·07	0·14	10	20·00	20·30
	0·16	20	2·00	2·14	0·12	20	20·00	20·65
Vanadium	0·20	0·5	2·00	2·01	0·16	0·5	20·00	19·96
	0·20	1	2·00	1·98	0·16	1	20·00	19·96
	0·19	3	2·00	1·98	0·16	3	20·00	20·06
	0·19	4	2·00	2·00	0·15	4	20·00	20·06
	0·19	5	2·00	2·05	0·15	5	20·00	20·08
	0·18	10	2·00	2·52	0·14	10	20·00	21·60
Copper	0·20	1	2·00	1·99	0·16	1	20·00	20·06
	0·19	5	2·00	2·02	0·15	5	20·00	19·96
	0·18	10	2·00	1·94	0·14	10	20·00	16·22

* Indistinct end-point.

Further tests were made to ascertain the effect of larger amounts of iron, chromium and nickel and metals known to interfere in other reduction methods used for the determination of tin. These tests established, as can be seen from Table II, that iron or nickel up to 20 per cent., tungsten up to 10 per cent. and copper, vanadium or molybdenum up to 5 per cent. have no significant effect on the determination of 2 and 20 per cent. of tin.

Interference from chromium depends on the amount of tin present. Table III shows that in the range 0·5 to 2 per cent. of tin, chromium in excess of 0·5 per cent. gave rise to erratic results, and in the determination of larger amounts of tin, chromium above 0·25 per cent. caused low results.

TABLE III

EFFECT OF CHROMIUM ON THE DETERMINATION OF TIN

Zirconium present, g	Chromium present, %	Tin	
		Added, %	Found, %
0·20	0·5	0·50	0·50
0·20	1·0	0·50	0·40
0·20	0·5	1·00	0·97
0·20	1·0	1·00	0·89
0·20	0·1	2·00	2·02
0·20	0·3	2·00	1·99
0·20	0·5	2·00	1·99
0·20	1·0	2·00	2·05
0·20	2·0	2·00	2·14
0·20	2·5	2·00	2·28
0·19	0·1	5·00	5·00
0·19	0·3	5·00	4·98
0·19	0·5	5·00	4·90
0·18	0·1	10·00	10·01
0·18	0·3	10·00	9·97
0·18	0·5	10·00	9·94
0·16	0·05	20·00	19·96
0·16	0·1	20·00	20·00
0·16	0·2	20·00	20·00
0·16	0·3	20·00	19·94
0·16	0·5	20·00	19·76
0·16	0·6	20·00	19·76

It was established that the effect of chromium can be overcome, in some instances, by diluting the sample solution to a known volume and taking an aliquot in order to reduce the amount of chromium in solution. Tests were carried out on two aliquots of a solution containing 0·14 g of zirconium and 20 mg of chromium (equivalent to 10·0 per cent.); the aliquots contained 1·0 mg of chromium and 2·0 mg of added tin (equivalent to 20·00 per cent.) and the tin found in each aliquot was 20·08 per cent.

When the chromium content is too large to permit this simple expedient, a preliminary separation of the tin with hydrogen sulphide is recommended. The results were satisfactory when solutions containing 5, 10 and 20 per cent. of chromium were examined in this way (see Table IV).

TABLE IV

DETERMINATION OF TIN BY THE HYDROGEN SULPHIDE PRECIPITATION METHOD

Zirconium present, g	Chromium present, %	Tin	
		Added, %	Found, %
0·19	5·0	2·00	1·98
0·15	20·0	2·00	1·98
0·15	10·0	12·00	11·97
0·12	20·0	20·00	19·88

Determination of tin below about 0·1 per cent.—Tests were made on solutions containing 0·005 to 0·05 per cent. of tin and 2 to 5 g of zirconium. To facilitate solution of the sample and prevent the formation of salts, the following acid mixtures were used—

Sample weight, g	2	5
Concentrated hydrochloric acid, ml ..	50	75
Fluoroboric acid, ml	10	15

In both instances the concentration of hydrochloric acid was adjusted to (1 + 1) before

reduction and the subsequent solutions were titrated with 0·005 *N* potassium iodate solution. The results were satisfactory for down to 0·01 per cent. of tin, as can be seen from Table V.

TABLE V

DETERMINATION OF SMALL AMOUNTS OF TIN

Zirconium present, g	Tin	
	Added, %	Found, %
2·0	0·050	0·050
2·0	0·050	0·049
5·0	0·010	0·0099
5·0	0·010	0·0097
5·0	0·005	0·0054

METHOD

The direct procedure should be used except when chromium is present in excess of 0·5 per cent. in the range 0·5 to 2 per cent. of tin or 0·25 per cent. in the range 2 to 20·0 per cent. of tin. When chromium exceeds these limits, use the fractionation procedure if practicable, otherwise use the hydrogen sulphide separation procedure.

REAGENTS—

Hydrochloric acid, concentrated, sp.gr. 1·18.

Hydrofluoric acid, 40 *per cent.*

Fluoroboric acid—To 280 ml of hydrofluoric acid (maintained at 10° C) add, in small quantities, 130 g of boric acid. Stir well and store in a polythene bottle.

Titanous chloride solution—Dissolve 5 g of titanium in 250 ml of concentrated hydrochloric acid, cool and dilute to 500 ml.

1 ml ≡ 10 mg of titanium.

Aluminium turnings, 99·5 *per cent. pure.*

Sodium bicarbonate solution—A 40 per cent. aqueous solution.

Potassium iodate solution, 0·02 *N*—Dissolve 0·7134 g of dry analytical-reagent grade potassium iodate and 10 g of potassium iodide in water, add 25 ml of 0·2 per cent. sodium hydroxide solution and dilute accurately to 1 litre. Standardise this solution against tin, in the presence of zirconium, by the recommended procedure.

1 ml ≡ 1·187 mg of tin (theoretical value).

Potassium iodate solutions, 0·01 and 0·005 *N*—Dilute the 0·02 *N* solution as required.

Sulphuric acid, diluted (1 + 4)—Carefully add 200 ml of concentrated sulphuric acid to 800 ml of water. Mix well and cool.

Nitric acid, concentrated, sp.gr. 1·42.

Cadmium sulphate solution, 10 *per cent.*—Dissolve 10 g of cadmium sulphate, $3CdSO_4.8H_2O$, in 100 ml of water.

Citric acid solution—A 60 per cent. aqueous solution.

Ammonium hydroxide, diluted (1 + 1)—Dilute 250 ml of ammonium hydroxide, sp.gr. 0·880, to 500 ml with water and mix well.

Sodium iodide.

Starch solution—A 1 per cent. aqueous solution.

DIRECT PROCEDURE—

Transfer a suitable weight of sample to a 500-ml conical flask and add the volumes of concentrated hydrochloric acid and fluoroboric acid specified in Table VI. Warm gently to assist solution, taking care to avoid undue loss of hydrochloric acid by evaporation. Adjust the concentration of acid to (1 + 1) with respect to hydrochloric acid, add 15 ml of titanous chloride solution, warm to about 70° C and add 1 g of aluminium turnings. When the aluminium has almost dissolved, insert a rubber bung provided with a delivery tube leading into sodium bicarbonate solution. Boil the sample solution gently for 10 minutes, and then cool it to room temperature with the outlet of the delivery tube maintained below the surface of the bicarbonate solution. Add 2 g of sodium iodide to the flask and replace the rubber

bung and delivery tube by an ordinary rubber bung or glass stopper. Shake the flask until the iodide has dissolved, add about 5 ml of starch solution and titrate with potassium iodate solution of suitable concentration, as follows—

Tin present, %	0·01 to 0·1	0·1 to 2·0	2·0 to 20·0
Potassium iodate solution required, *N* ..	0·005	0·01	0·02

FRACTIONATION PROCEDURE—

Dilute the sample solution, obtained as for the direct procedure, to 250 ml and take an aliquot in order to bring the chromium content to within the limiting values. Adjust the acidity to (1 + 1) with respect to hydrochloric acid, add 15 ml of titanous chloride solution and continue as described for the direct procedure.

HYDROGEN SULPHIDE SEPARATION PROCEDURE—

Dissolve 0·2 g of the sample in 40 ml of diluted sulphuric acid (1 + 4) and 5 ml of fluoroboric acid; warm gently to assist solution. Oxidise with a slight excess of concentrated nitric acid, added dropwise, and then boil for 2 to 3 minutes to remove nitrous fumes and ensure complete solution of the tin. Cool, dilute to about 250 ml, and add 2 ml of cadmium sulphate solution and 20 ml of citric acid solution. Neutralise with diluted ammonium hydroxide (1 + 1), and then acidify with 25 ml of diluted sulphuric acid (1 +4). Dilute to about 400 ml, warm to about 80° C and pass hydrogen sulphide through the solution for 30 minutes.

Set the mixture aside for at least 3 hours, and then collect the precipitate on a Whatman No. 42 filter-paper and wash it once with cold water containing hydrogen sulphide. Dissolve the precipitate from the paper with 50 ml of warm concentrated hydrochloric acid, into a 500-ml conical flask and wash the paper with about 50 ml of water. Add 15 ml of titanous chloride solution and continue as described for the direct procedure.

TABLE VI

VOLUMES OF HYDROCHLORIC AND FLUOROBORIC ACIDS REQUIRED FOR VARIOUS AMOUNTS OF TIN

Tin present, %	Sample weight, g	Solvent composition: Hydrochloric acid, concentrated, ml	Solvent composition: Fluoroboric acid, ml
0·5 to 20·0	0·2	50	5
0·1 to 0·5	1	50	5
0·05 to 0·1	2	50	10
0·01 to 0·05	5	75	15

RESULTS

Results of the determination of tin in five different samples, which are given by permission of the Managing Director, United Kingdom Atomic Energy Authority, Industrial Group, and relate to work carried out on these samples by the recommended and other procedures, in the laboratories of Chemical Services Department, U.K.A.E.A., Springfields Works, Salwick, were as follows—

Tin found by sulphide - hypophosphite method, %	1·60	1·40	1·45	1·45	1·40
Tin found by polarographic method, %	1·54	1·38	1·44	1·49	1·39
Tin found by recommended method, direct procedure, % ..	1·55	1·39	1·43	1·40	1·37

CONCLUSIONS

The direct procedure is suitable for the determination of 0·01 to 20 per cent. of tin in zirconium and its alloys.

The procedure can also be extended to the determination of larger amounts of tin by taking a suitable aliquot of the sample solution. It is simple and rapid and about thirty samples can be analysed in 8 hours by one analyst. It is, therefore, particularly suitable for control analysis, and has been satisfactorily applied on a routine basis to samples of Zircalloy

2, which contain 1·5 per cent. of tin, and to zirconium master alloys containing 40 per cent. of tin and 2·5 per cent. of chromium.

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Separation of Niobium from Tantalum, Titanium, Tin and Antimony by means of 8-Hydroxyquinoline

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From solutions of earth acids in tartrate medium and adjusted to pH 6·0, niobium can be quantitatively precipitated by means of 8-hydroxyquinoline. From the filtrate, tantalum can be recovered by means of tannic acid. Titanium, when present together with earth acids, is distributed in the two fractions, the major amount remaining with the tantalum. Each fraction is corrected for titanium after determining it by the peroxide method.

For oxides obtained by a direct ethylenediaminetetra-acetic acid - tannic acid procedure and containing tin and antimony oxides, the same procedure for the separation of niobium in the tartrate medium is followed. However, before the recovery of tantalum by tannic acid, tin and antimony in the filtrate after treatment with 8-hydroxyquinoline are removed by precipitation as sulphides with hydrogen sulphide.

Owing to the very close similarity in their chemical properties, the separation of niobium from tantalum and other earth acids has always proved to be a laborious task. Marignac[1] and others[2,3,4,5] separated tantalum from obinium as their fluoro salts. Gillis *et al.*[6] separated the tantalum - ferroin complex in hydrochloric acid solution. Powell and Schoeller[7] introduced the use of tannic acid for separating these elements and this involved several fractional precipitations after prior removal of titanium. Several workers[8,9,10,11,12] have attempted to simplify the Schoeller method. Chromatographic methods of separation have been developed by Burstall, Swain, Williams and Wood,[13] and by others,[14,15] in which the fluoro salts of niobium and tantalum are extracted with ethyl methyl ketone with different concentrations of hydrofluoric acid. Ion-exchange methods[16,17,18] include the use of oxalic acid, or a mixture of hydrochloric and hydrofluoric acids, and Dowex resins. Other methods used for separation include the use of sodium hypophosphite[19] for complexing tantalum; taking advantage of the solubility of hydrated niobium oxide in concentrated sulphuric acid[20]; electrolytic separation of niobium[21,22]; separation of tantalum with potassium carbonate[23]; leaching of the bisulphate melt of earth acids with oxalic acid and ammonium oxalate[24]; extraction of niobium in concentrated hydrochloric acid with methyldioctylamine[25] or tribenzylamine[26]; precipitation of tantalum as its iodate complex[27]; and reduction of niobium with selenium oxychloride,[5] to leave the tantalum compound insoluble. Besides these chemical methods, some physico-chemical methods are also available in which chlorination and distillation techniques are involved.[28,29]

All these methods have their own merits; but most of them involve complicated procedures and have therefore proved tedious. In all the methods described above for the separation of niobium and tantalum, the earth acids have to be first obtained by one of the

standard procedures such as the one described by Schoeller. Recently, Sankar Das, Venkateswarlu and Athavale[30] have described a modification to the Schoeller tannic acid procedure in which the earth acids and titania are separated with tannic acid in the presence of ethylenediaminetetra-acetic acid. In this method, however, tin and antimony, if present in the mineral, are also quantitatively precipitated with the earth acids.

Sue[31] has shown that niobium can be determined with 8-hydroxyquinoline (oxine) in neutral solutions containing ammonium oxalate and ammonium acetate. Schwarz[32] investigated the possibility of using oxine for the separation of niobium from tantalum, but without any important results. Cattelain[33] has shown that tin and antimony do not form insoluble complexes with oxine in tartrate medium, and tungsten is incompletely precipitated.[34] Jilek and Rysanek[35] precipitated tungsten from an oxalate - ammonium acetate solution with oxine in solutions neutral to methyl red (at pH 6·3).

The present work aims at the separation of niobium from tantalum and titanium, and also from tin and antimony, by means of oxine.

Experimental

Materials—

A Marconi bench-type pH meter, measuring to ± 0·02, was used. Niobium and tantalum stock solutions were prepared from niobium pentoxide (free from tantalum and titanium) and tantalum pentoxide (free from niobium and titanium), supplied by A. D. Mackey, Inc. The titanium stock solution was prepared from Specpure titanium oxide. Weighed amounts of the oxides, ignited previously to 1000° C for 2 hours, were fused separately with potassium hydrogen sulphate, and the melt was dissolved in known amounts of either ammonium oxalate or ammonium tartrate solution, as was necessary for the various sets of experiments, and diluted to known volumes, so that 1 ml ≡ 1 mg of oxide. This was further checked by the Schoeller tannic acid method.

A 4 per cent. w/v solution of analytical-reagent grade 8-hydroxyquinoline in 90 per cent. ethanol was used and was adjusted to the desired pH.

All other reagents used were of analytical-reagent grade.

Preliminary work—

With oxine as a precipitating agent at pH 6·5, a few preliminary experiments were carried out to determine the conditions of precipitation of niobium, tantalum and titanium separately, in an oxalate and a tartrate medium. The results are shown in Table I.

Table I

Determination of niobium, tantalum and titanium in various media

Medium	Element	Amount added, as oxide, mg	Amount found, as oxide, mg	Amount recovered by means of tannic acid from the filtrate, as oxide, mg
Solution containing 2 per cent. w/v of oxalate ion	Nb	40·0	40·0	n.d.
	Ta	25·0	23·8	n.d.
	Ti	25·0	24·7	n.d.
	Nb + Ta	44·0	44·5	n.d.
Solution containing 2 per cent. w/v of both oxalate and tartrate ion	Nb + Ta	44·0	35·4	n.d.
Solution containing 2 per cent. w/v of tartrate ion	Ta	50·0	0·2	50·2
	Nb	50·0	50·2	0·2
	Nb + Ta	50·0 + 50·0	51·4	49·3

n.d. = not determined.

The results for the medium containing oxalate ions in Table I indicate that niobium and titanium are completely, and tantalum almost completely, precipitated by oxine at pH 6·5. It is also seen that the presence of tartrate ions together with the oxalate ions interferes seriously in the precipitation of these metals as their oxinates. The results for the medium containing tartrate ions indicate clearly that these ions, when present alone, almost prevent the precipitation of tantalum and give a nearly quantitative precipitation of niobium as its

oxinate. The tantalum, in this instance, can be recovered quantitatively from the filtrate by means of tannic acid.

Hence, a separation of niobium from tantalum was found to be feasible in the tartrate medium alone and so further experiments were carried out with this medium.

EFFECT OF pH ON PRECIPITATION BY OXINE—

During the experiments described above, it was observed that the precipitation of niobium with oxine started at pH 4·3 and was complete even at pH 6·5. Tantalum, in the tartrate medium, was not precipitated with oxine even up to pH 8·0, whereas complete precipitation of titanium took place above pH 6·5. Titanium, however, gets distributed in both the fractions, *viz.*, in the oxinate precipitate and the filtrate. If the pH were kept below 6·0, most of the titanium would remain in the oxine filtrate, to be recovered with tantalum. It was, however, felt that if the pH is too low, conditions may be set up more favourable for the hydrolysis of tantalum and thereby contaminate the niobium oxinate. A pH of about 6·0 was therefore considered to be more suitable.

In all the subsequent experiments it was found that a double precipitation of the oxinate was necessary to remove the adsorbed metals. The presence of oxine did not interfere with the recovery of metals from the filtrate with tannic acid. Titanium was determined and was allowed for in each fraction, after ignition, by the usual hydrogen peroxide method.

PROCEDURE—

Fuse the purified oxides (with or without tin and antimony oxides) weighing not more than 0·2000 g (or preferably 0·1000 g) with 8 to 10 g of sodium hydrogen sulphate, thoroughly to the crystallisation point. Extract the cooled melt with 50 ml of saturated ammonium tartrate solution for about ½ hour on a water bath, add 100 ml of distilled water containing 5 ml of ammonia solution, sp.gr. 0·90, and continue heating with occasional stirring, until the solution is clear. Filter, if necessary, and repeat the procedure after igniting the insoluble matter. Dilute the clear solution to about 400 ml. Add more ammonium tartrate solution so as to bring up its concentration to about 2 per cent. w/v. Next add 8 g of ammonium chloride and adjust the pH of the solution to 6·0. Warm the solution on a water bath and add 5 to 10 ml of oxine solution (of pH 6·0) until the precipitation is complete. Digest the precipitate on the water bath for ½ hour more with occasional stirring. Add filter-pulp and cool for about 10 minutes. Filter through Whatman No. 42 15-cm filter-paper and wash the precipitate free from sulphate with a cold wash solution (of pH 6·0) containing 0·1 per cent. of oxine and 1 per cent. each of ammonium tartrate and ammonium chloride. Transfer the filter-paper containing the precipitate to a weighed platinum crucible, cover it with 1 to 2 g of oxalic acid crystals to prevent escape of the volatiles oxinates, dry, and ignite and weigh in the usual manner. Follow the same procedure as described above for carrying out a re-precipitation. Since the oxides obtained need purification, treat with a few millilitres of hydrofluoric and a few drops of concentrated sulphuric acid, evaporate to fumes of sulphur trioxide, ignite and treat the oxides with dilute hydrochloric acid, if necessary, to make free from soluble impurities.

Recover tantalum and titanium from the filtrate with tannic acid in the usual manner and purify the oxides as indicated above.

As the original oxides may contain tin and antimony oxides, saturate the slightly acidic filtrate after the oxine precipitation with hydrogen sulphide to separate the insoluble sulphides of tin and antimony, filter, wash with water containing hydrogen sulphide and tartrate ions. Boil the filtrate and washings to expel hydrogen sulphide and precipitate tantalum and titanium with tannic acid as described above.

RESULTS

SEPARATION OF BINARY AND TERNARY MIXTURES—

Some binary and ternary mixtures of earth acids and titania as well as mixtures of earth acids with tin and antimony oxides were analysed by the proposed method.

Results obtained with binary and ternary mixtures of niobium, tantalum and titanium are given in Table II. The results show that the separations are reasonably good in the whole range of Ta_2O_5 to Nb_2O_5 ratios from 20·0 to 0·05. A slight positive error, more or less, of a constant nature in both the oxine and tannic acid fractions is indicated in most of the

experiments. Calculated, however, on the basis of the total oxides taken in each experiment, this error is not more than 1·5 per cent. for any fraction.

Table II

Binary and ternary mixtures of niobium, tantalum and titanium

Oxide taken				Oxine fraction			Tannic acid fraction		
Ta_2O_5, mg	Nb_2O_5, mg	TiO_2, mg	Ratio of Ta_2O_5 to Nb_2O_5,	Nb_2O_5 found (corrected for TiO_2), mg	Difference, mg	TiO_2 found, mg	TiO_2 found, mg	Ta_2O_5 (corrected for TiO_2), mg	Difference, mg
50·0	0·0	5·0	—	—	—	0·5	4·2	50·7	+0·7
0·0	50·0	5·0	—	50·0	Nil	0·7	4·0	—	—
100·0	100·0	0·0	1·0	100·2	+0·2	—	—	100·3	+0·3
50·0	50·0	0·0	1·0	50·2	+0·2	—	—	50·5	+0·5
40·0	40·0	5·0	1·0	39·1	−0·9	0·3	4·8	40·9	+0·9
40·0	20·0	5·0	2·0	20·3	+0·3	0·2	4·8	40·8	+0·8
40·0	16·0	5·0	2·5	16·3	+0·3	0·2	4·8	40·8	+0·8
40·0	8·0	5·0	5·0	8·5	+0·5	0·2	4·8	40·6	+0·6
40·0	4·0	5·0	10·0	3·5	−0·5	0·2	4·6	40·2	+0·2
40·0	2·0	5·0	20·0	2·3	+0·3	0·1	4·8	40·5	+0·5
20·0	40·0	5·0	0·5	40·2	+0·2	0·3	4·8	20·9	+0·9
16·0	40·0	5·0	0·4	40·0	0·0	0·2	4·6	16·8	+0·8
8·0	40·0	5·0	0·2	40·2	+0·2	0·2	4·8	8·7	+0·7
2·0	40·0	5·0	0·05	40·0	0·0	0·3	4·6	2·4	+0·4

Table III gives the results of some experiments in which tin and antimony oxides were added to the earth acids and separations were carried out in the manner described under "Procedure."

Hence, the method works extremely well for the separation and determination of the metals tried. Tin and antimony, if present, have to be determined in the sulphide precipitate by the usual methods.

Purity of the niobium and tantalum fractions—

It is not reasonable to expect in a mixture of earth acids a separation of any element spectrographically free from the other. A semi-quantitative spectrographic examination of a few oxine and tannic acid fractions showed that there was contamination of the niobium fraction with tantalum and *vice versa*, but the purity of each fraction was better than 97·5 per cent.

Table III

Influence of associated elements

Sample	Elements	Oxide taken, mg	Oxides found by the oxine precipitation (Nb_2O_5), mg	Oxides found by the tannic acid after separation with H_2S (Ta_2O_5), mg	Error, mg
A	Nb	25·0	25·2	—	+0·2
	Ta	22·0	—	22·1	+0·1
	Sn	50·0	—	—	—
B	Nb	25·0	25·4	—	+0·4
	Ta	22·0	—	21·8	−0·2
	Sb	48·0	—	—	—
C	Nb	25·0	25·2	—	+0·2
	Ta	22·0	—	21·6	−0·4
	Sn	25·0	—	—	—
	Sb	32·0	—	—	—
D	Ta	22·0	—	21·8	−0·2
	Sn	25·0	—	—	—
E	Ta	22·0	—	21·6	−0·4
	Sb	32·0	—	—	—

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A Rapid Method for the Determination of Calcium and Magnesium in Plant Material by Titration with Disodium Ethylenediaminetetra-acetate

BY V. P. PADHYE

Rapid removal of interfering ions from the extracts of plant ash enables improved end-points to be obtained in the determination of calcium and magnesium by titration with disodium ethylenediaminetetra-acetate without the introduction of time-consuming purification procedures. Phosphate is removed by precipitation with zirconium nitrate and iron, manganese and traces of the heavy metals are extracted with carbon tetrachloride as their diethyldithiocarbamate complexes. The results obtained by the method compare favourably with those obtained by conventional methods and recovery of added calcium and magnesium is excellent.

THE use of the disodium salt of ethylenediaminetetra-acetic acid (EDTA) for the determination of calcium and magnesium in plant material is now an accepted technique. In establishing the method, a considerable amount of research has been directed towards

improving the sharpness and reproducibility of the end-points by prior removal of interfering ions. In extracts of plant ash, iron, manganese and phosphate ions are usually present in sufficient amounts to mask the true end-points; heavy-metal ions will, in theory, also interfere, although in common plant material they are seldom present in sufficient amounts to do so. Many suggestions for the removal of these ions have been made. Cheng and Bray[1] investigated the limits of interference of iron, manganese, aluminium and phosphate and gave a method for the removal of the sesquioxides and manganese by oxidation, precipitation and filtration. Removal of heavy metals can be effected by treatment with sodium diethyldithiocarbamate and subsequent extraction, either with *iso*amyl alcohol and chloroform, as suggested by Cheng, Melsted and Bray,[2] or with carbon tetrachloride, as suggested by Forster.[3] Other refinements that have been described are the precipitation of calcium as its oxalate, followed by the determination of magnesium in the filtrate[3] or the removal of interfering ions by cation-exchange resins.[4] Smith and McCallum[5] have investigated the nature of the interference of iron, manganese, phosphate and ammonium ions, and the limits of their interference. They described the removal of phosphate by double precipitation as ferric phosphate at pH 3 to 4, followed by the removal of manganese and excess of iron by precipitation with ammonium hydroxide after oxidation with bromine water.

The accuracy of the conventional methods, namely the precipitation of calcium as the oxalate and magnesium as the 8-hydroxyquinolate, cannot be seriously questioned. Both methods, however, and particularly that for magnesium, are time-consuming. Although EDTA titrations offer a means of increasing the speed with which these two ions may be determined, the difficulties inherent in detecting the true end-points detract considerably from the value of the reagent, and it seems that efforts to improve the accuracy of detecting the end-points have so far led to methods of purification equally as time-consuming as those that are necessary in the classical methods. This is most apparent in the determination of calcium.

This paper presents a method that aims at obtaining sharp and reproducible end-points by the prior removal of interfering ions, but in which the methods of removal are such that the advantages of speed are retained. In the opinion of the author it is only if this aim is achieved that the EDTA method for the determination of calcium and magnesium in plant material becomes a worthy rival to the classical methods.

Experimental

Removal of phosphate—

Precipitation of phosphate by the addition of excess of ferric chloride involves careful control of pH. The ammonium ions subsequently added to remove the excess of iron must be destroyed before a titration in which murexide indicator is used can be carried out. Zirconium nitrate solution will precipitate phosphate quantitatively in dilute acid solution and this method requires a minimum of control and manipulation; the excess ions introduced do not interfere with the subsequent titrations. The addition of 1 ml of a 2 per cent. aqueous solution of zirconium nitrate was found to be adequate for the complete precipitation of the phosphate in 1 g of plant material.

To examine the effect, if any, of zirconyl ions on the EDTA titrations, a solution containing calcium, magnesium and phosphate was prepared, the amounts of the three ions present being of the same order as might be expected in the extract of an average plant ash. The amounts of calcium, magnesium and phosphate present in the solution were determined by conventional methods and 100 ml of solution were found to contain 39·49 mg of calcium, 15·40 mg of magnesium and 20·0 mg of phosphorus. Calcium and magnesium were then determined in this solution by the EDTA method (*a*) without removal of phosphate and (*b*) with prior removal of phosphate by precipitation with zirconium nitrate solution.

No satisfactory end-point could be obtained for titrations with EDTA in which murexide indicator was used in the presence of this amount of phosphate. Consequently the accurate determination of both calcium and magnesium was impossible. However, after removal of phosphate, eight determinations of calcium in 100 ml of solution had a recovery range of 39·22 to 39·39 mg, a mean recovery of 39·26 mg and a standard deviation of $\pm 0{\cdot}063$; the recovery was 99·9 per cent. Eight determinations of magnesium in the same solution had a recovery range of 15·53 to 15·73 mg, a mean recovery of 15·65 mg and a standard deviation of $\pm 0{\cdot}065$; the recovery was 101·6 per cent.

REMOVAL OF IRON AND MANGANESE—

Treatment with sodium diethyldithiocarbamate can be used to remove iron and manganese and traces of heavy metals.[2] This is considerably more rapid than precipitation methods. During investigation of the method, low recoveries of calcium were often obtained with synthetic solutions. It was also observed that after extraction of the diethyldithiocarbamate complexes with carbon tetrachloride, the solution remaining in the separating funnel was turbid. The solution could be cleared by filtration and the turbidity was shown to be due to the precipitation of the relatively insoluble calcium salt of the extracting reagent. It was found that this only occurred if an excess of sodium diethyldithiocarbamate was added. This is easily done if the solid salt is added, as recommended in most methods reported in the literature, but can be avoided if only 1 ml of a 1 per cent. aqueous solution of the salt is added at a time. This involves the preparation of a fresh solution of the carbamate reagent for each batch of determination, but it is considered that this is worth while, since the risk of losing calcium by precipitation is eliminated.

REAGENT BLANK—

A blank of the reagents should be carried through all the stages of the method and used as a standard for comparison in determining the end-point of the titration with murexide indicator. This was found to be of greater assistance than the various synthetic colour standards that have been suggested from time to time.

METHOD

REAGENTS—

EDTA solution—Dissolve 8·25 g of disodium ethylenediaminetetra-acetate in 46 ml of *N* sodium hydroxide solution and make up to approximately 2·5 litres with water. Standardise the solution against 10 ml of the standard calcium solution to which 2 ml of a 10 per cent. solution of potassium hydroxide had been added.

Standard calcium solution—Dissolve 1·2486 g of dried calcium carbonate in 5 ml of diluted hydrochloric acid (1 + 1) and make up to 500 ml with water. This solution must be standardised gravimetrically and should contain 1 mg of calcium per ml.

Standard magnesium solution—Dissolve 5·0700 g of magnesium sulphate, $MgSO_4.7H_2O$, in water and make up to 500 ml. This solution contains 1 mg of magnesium per ml.

Murexide indicator—Mix thoroughly 40 g of potassium sulphate and 0·2 g of murexide. Use 2 ml of a 2 per cent. aqueous solution of this powder, freshly prepared.

Eriochrome black T indicator solution—Dissolve 0·5 g of the reagent in a mixture of 1 ml of *N* sodium carbonate solution and 30 ml of *iso*propanol and make up to 100 ml with distilled water.

Zirconium nitrate solution, 2 per cent. w/v—Suspend 2 g of zirconium nitrate in water and add a few drops of concentrated nitric acid, warm until dissolved, then filter and make up to 100 ml with water.

Sodium diethyldithiocarbamate solution, 1 per cent. w/*v*—Prepare this aqueous solution as required and filter before use.

Potassium hydroxide solution, 10 per cent. w/v.

Buffer solution at pH 10—Dissolve 67·5 g of ammonium chloride in about 200 ml of water. Add 570 ml of concentrated ammonia solution and make up to 1 litre with water.

PROCEDURE FOR PREPARING EXTRACT OF PLANT ASH—

Place 5 g of the dried ground plant material in a suitable glazed silica crucible or dish, add sufficient ethanol to cover the sample and ignite. When flaming has ceased, rake up the sample to expose the unburnt portion, and wet it with ethanol and re-ignite. Repeat this until the whole sample is carbonised. Then ignite the sample over a small bunsen flame until the ash turns greyish and then place it in a muffle furnace at 450° to 550° C until the ash is almost white. Remove the crucible from the furnace, cool it and wet the ash with *N* sodium nitrate solution. Dry the treated ash, first over a boiling-water bath and then gently over a burner flame. Repeat the sodium nitrate and drying treatment until the ash is white and then return the crucible to the muffle furnace at 450° to 550° C for 5 minutes.

Remove the crucible from the furnace, cool it and cover it with a watch-glass, and add 10 ml of 6 *N* hydrochloric acid, or at least sufficient acid to wet the ash thoroughly. Place

the crucible, still covered, on a boiling-water bath and allow the ash to digest for 20 minutes. Remove, rinse down the watch-glass with distilled water and evaporate the contents of the crucible to dryness. When dry, heat for an hour in the oven at 110° C to complete the dehydration of silica.

Add about 10 ml of 0·1 *N* nitric acid to the ash, warm and stir thoroughly to dissolve all the soluble salts. Filter into a 25-ml calibrated flask, transferring the insoluble residue of the ash to the filter-paper. Wash out the crucible and wash the filter-paper with successive small portions of warm 0·1 *N* nitric acid until the volume of the filtrate is just under 25 ml. Allow it to cool and make up to 25 ml with 0·1 *N* nitric acid and mix thoroughly.

By pipette, transfer 5 ml of the solution of the ash to a 15-ml centrifuge tube, add 1 ml of 2 per cent. zirconium nitrate solution, mix thoroughly and place the centrifuge tube in a warm-water bath for 20 minutes to aid flocculation of the precipitate. Centrifuge at about 2000 r.p.m. for 10 minutes. Pour the supernatant liquid into a separating funnel. Wash the residue with a few millilitres of cold water, re-centrifuge and add the supernatant liquid to the solution in the separating funnel. Add 1 ml of 1 per cent. sodium diethyldithiocarbamate solution, shake the separating funnel and add a few millilitres of carbon tetrachloride, shake the funnel again and allow the layers to separate. Run off the carbon tetrachloride layer and repeat the extraction procedure with further small portions of carbon tetrachloride until the organic solvent layer is colourless. Two extractions are usually sufficient. Then add 1 or 2 drops more (do not add an excess at this stage) and repeat the extraction once more to ensure that all traces of iron and manganese are removed. Filter the aqueous solution through a plug of cotton-wool into a 25-ml calibrated flask, wash the plug and funnel thoroughly with distilled water and make the solution up to the mark.

PROCEDURE FOR DETERMINING CALCIUM—

To a 10-ml aliquot of the solution add 2 ml of 10 per cent. potassium hydroxide solution and 2 ml of the murexide indicator solution and titrate with standardised EDTA solution until the colour changes from pink to purple and matches that of the blank.

PROCEDURE FOR DETERMINING MAGNESIUM—

To another 10-ml aliquot of the solution add 5 ml of the buffer solution at pH 10 and 4 drops of Eriochrome black T indicator solution and titrate with standardised EDTA solution until the colour changes from wine-red to blue. The volume of EDTA solution equivalent to the magnesium present is equal to the volume used for the magnesium titration minus the volume used for the calcium titration.

RESULTS

Calcium and magnesium were determined in samples of plant leaves by conventional methods and by the proposed method. The results are shown in Table I.

TABLE I

DETERMINATION OF CALCIUM AND MAGNESIUM IN VARIOUS PLANT LEAVES BY DIFFERENT METHODS

Sample	Calcium found by oxalate - permanganate method, %	Calcium found by proposed method, %	Magnesium found by oxine method, %	Magnesium found by proposed method, %
Banana A	0·43	0·38	0·32	0·28
Banana B	0·35	0·31	0·31	0·29
Coffee A	0·60	0·56	0·27	0·25
Coffee B	0·67	0·63	0·35	0·33
Cashew A	0·17	0·16	0·16	0·15
Cashew B	0·15	0·16	0·18	0·17
Cashew C	0·079	0·078	0·19	0·18
Cashew D	0·086	0·089	0·20	0·17
Wattle A	0·44	0·42	0·15	0·145
Wattle B	0·38	0·36	0·14	0·13
Wattle C	0·31	0·30	0·14	0·13
Pine A	0·46	0·49	0·20	0·18
Pine B	0·42	0·41	0·16	0·15
Grass	0·36	0·37	0·14	0·17

The recovery of calcium and magnesium added to some extracts of plant ash was determined by the proposed method, with the results shown in Table II.

Table II

Recovery of calcium and magnesium added to extracts of plant ash

Calcium—

Sample	Calcium in extract, mg	Calcium added, mg	Calcium found, mg	Recovery of added calcium	
				mg	%
Cashew	1·59	0·75	2·35	0·76	101·3
Wattle A	4·24	2·00	6·31	2·07	103·5
Wattle B	3·63	1·80	5·45	1·82	101·1
Pine	1·56	0·90	2·51	0·95	105·6
Coffee	6·35	3·30	9·67	3·32	100·6
Banana	3·08	1·70	4·84	1·76	103·5

Magnesium—

Sample	Magnesium in extract, mg	Magnesium added, mg	Magnesium found, mg	Recovery of added magnesium	
				mg	%
Cashew	1·70	1·00	2·72	1·02	102·0
Wattle A	1·45	0·80	2·27	0·82	102·5
Wattle B	1·33	0·80	2·11	0·78	97·5
Pine	0·72	0·40	1·11	0·39	97·5
Coffee	3·36	1·90	5·33	1·97	103·7
Banana	2·92	1·70	4·64	1·72	101·2

Conclusions

The methods employed to remove interfering ions enable the most to be made of the EDTA method of determining calcium and magnesium in extracts of plant ash. The method is more rapid than the conventional methods, but compares very favourably with them in accuracy and reproducibility. It is therefore suitable for laboratories that have to deal with a fairly large number of samples, but where a reasonable degree of accuracy is required.

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The Semi-micro Determination of Phosphorus in Fluorinated Organic Compounds

By T. R. F. W. FENNELL, M. W. ROBERTS and J. R. WEBB

Two methods for the semi-micro determination of phosphorus in fluorinated organic materials have been investigated. It was found that wet-oxidation with nitric and sulphuric acids was not universally applicable, but that decomposition by a modified fusion with sodium peroxide, followed by precipitation of quinoline molybdophosphate was a reliable, rapid and accurate method. This procedure is described in detail. Results obtained are shown to be within $\pm 0{\cdot}15$ per cent. absolute.

As a result of investigations being carried out in these laboratories into the preparation and properties of fluorinated materials, a method was required for the determination of phosphorus in fluorinated organic compounds.

Numerous papers have been published on the determination of phosphorus in unfluorinated organic materials, and a review of the methods adopted is given in the introduction of a paper by Burton and Riley.[1] There appears to be little mention of the analysis of fluorinated materials. Bennett, Emeleus and Haszeldine[2] determined phosphorus in a limited range of materials after hydrolysis with aqueous alkali, and Rush, Cruickshank and Rhodes[3] mentioned the use of the Carius method in the analysis of fluorocarbon compounds.

Experimental

Wet-oxidation method—

The method of Belcher and Godbert,[4] in which phosphorus is determined gravimetrically as nitratopentamminecobaltidodecamolybdophosphate after wet-oxidation with nitric and sulphuric acids, was found to give very good results for the determination of phosphorus in unfluorinated compounds. Contrary to the experience of Burton and Riley,[1] we found no difficulty in the breakdown of triphenylphosphine. As special apparatus is not required in this method, we carried out tests with mixtures of inorganic fluoride and phosphate salts and with organic fluorine-containing and phosphorus-containing compounds and found that good results were obtained without special precautions. Furman and State[5] reported low results in the presence of fluoride and after fuming with sulphuric acid to remove fluoride.

The method was then applied to the analysis of some fluorinated research compounds. Good results were obtained for some of these, but not for others. Typical results are shown in Table I.

Table I

Results by wet-oxidation method

Compound	Phosphorus calculated, %	Phosphorus found, %
$(CH_3{\cdot}C_6H_4{\cdot}O)_2P(O)NH{\cdot}C_6H_5$	8·78	8·85
$(CF_3{\cdot}CH_2{\cdot}O)_2P(O)NH{\cdot}C_6H_5$	9·19	9·04
$C_2F_7{\cdot}CH_2{\cdot}O{\cdot}P(O)(NH{\cdot}C_6H_5)_2$	7·20	7·22
$C_2F_7{\cdot}CH_2{\cdot}O{\cdot}P(O)(NH{\cdot}C_6H_4{\cdot}CH_3)_2$	6·76	6·79
$(C_2F_7{\cdot}CH_2{\cdot}O)_2P(O)NH{\cdot}C_6H_5$	5·77	3·00
$(C_2F_7{\cdot}CH_2{\cdot}O)_2P(O)NH{\cdot}C_6H_4{\cdot}CH_3$	5·62	3·11
$(C_2F_7{\cdot}CH_2{\cdot}O)_3PO$	4·81	Nil

Peroxide bomb method—

After the wet-oxidation method had proved partly unsuccessful, we investigated the peroxide bomb method. Determination of the resulting phosphate was carried out by Wilson's method.[6] Preliminary work on inorganic standards had shown that this method (originally designed to deal with about 20 mg of phosphorus) could be used, with modification, for the determination of 2 to 3 mg of phosphorus. It was more rapid than the precipitation of the cobalt complex.

It was found, however, that relatively large and variable blank values were obtained when a semi-micro (8·5-ml capacity) nickel bomb and the usual amounts of reagents[7,8] for semi-micro operation were used, *i.e.*, 4 g of sodium peroxide, 200 mg of potassium nitrate and the organic material made up to 200 mg with sucrose. These blank values were caused by silica picked up from the glass apparatus used during the weighing of the sodium peroxide and during the leaching of the bomb, and they could be eliminated by using platinum apparatus for these operations, the bomb leachings being transferred to glass apparatus only after acidification.

We could not conveniently leach the residue from the semi-micro bomb in the available platinum basins, which would, however, accommodate a micro (3 ml capacity) bomb. Tests were carried out in this micro bomb with 25 mg of standard compounds, 25 mg of sucrose, 50 mg of potassium nitrate and 1 g of sodium peroxide. The results obtained, which are shown in Table II, indicated that fusion in the smaller bomb was satisfactory.

TABLE II

ANALYSIS OF STANDARD COMPOUNDS BY MICRO BOMB FUSION METHOD

Compound	Number of determinations	Phosphorus calculated, %	Mean phosphorus found, %	Deviation from mean
Triphenylphosphine	6	11·81	11·80	±0·14
Tri-*n*-butyl phosphate	4	11·63	11·69	±0·14
Tri-*m*-cresyl phosphate ..	5	8·41	8·42	±0·04

It was found that the presence of fluoride in the bomb leachings gave rise to positive errors in determinations of phosphorus, presumably by attack on the glass flask. Interference by fluoride could be overcome by evaporating the bomb leachings, acidified with hydrochloric acid, to dryness two or three times, or by adding boric acid.

METHOD

REAGENTS—

All reagents should be of recognised analytical grade.

Sodium peroxide.

Sucrose.

Boric acid.

Potassium nitrate.

Hydrochloric acid, concentrated, dilute (1 + 9) *and* 0·5 N.

Sodium hydroxide, 0·5 N—This solution must be stored in a polythene bottle.

Sodium molybdate solution—Dissolve 15 g of the hydrated salt by shaking with 100 ml of water in a polythene flask. Set aside for 24 hours and filter into a polythene flask.

Quinoline hydrochloride solution—Dissolve 20 ml of redistilled quinoline in 800 ml of hot water containing 25 ml of concentrated hydrochloric acid. Stir well, cool to room temperature and filter through a paper-pulp pad. Do not wash the pad, but make the filtrate up to 1 litre.

Mixed indicator solution—Mix 2 volumes of 0·1 per cent. phenolphthalein solution with 3 volumes of 0·1 per cent. thymol blue solution (both in ethanol).

PROCEDURE—

Weigh into the cup of the bomb an amount of organic material that contains 2 to 3 mg of phosphorus. Add sucrose to make the total organic material up to 50 mg and 50 mg of potassium nitrate. The potassium nitrate should be placed in the bomb cup first if a liquid substance is to be analysed. Weigh, on platinum foil, 1 g of sodium peroxide and brush it into the bomb.

Secure the cap, lightly tap the side of the bomb to mix the contents, and heat the lower part of the cup for not more than 30 seconds in the tip of a micro-burner flame. Allow the bomb to cool for 10 minutes and then quench it in distilled water. Remove the stirrup and

wipe the exterior of the bomb with a clean towel. Hold the bomb over a platinum basin (or crucible) of sufficient capacity, *i.e.*, having a depth of 3 to 4 cms. Remove the cap and place the cup in the basin. Wash the inner surface of the cap with a jet of water, allowing the washings to fall into the basin. Add sufficient water to half fill the bomb as it lies on its side. Cover the basin immediately with a watch-glass and warm it on a hot-plate until the contents dissolve. After about 5 minutes, leaching is complete.

Raise the watch-glass and wash the under surface with water into the basin. Remove the bomb cup with nickel forceps or tongs, washing the inner and outer surfaces with water. Replace the watch-glass and warm the basin on a hot-plate for a few minutes to drive oxygen out of solution.

When effervescence ceases, cool the basin, raise the edge of the watch-glass and add two drops of mixed indicator solution. (A lip on the platinum basin is convenient, as the watch-glass can then be moved slightly aside to expose the lip.) Cautiously add concentrated hydrochloric acid from a dropping pipette through the lip, allowing the effervescence to cease between additions, until the indicator changes through yellow just to pink. Wash the tip of the pipette with water into the basin. Replace the basin on the hot-plate and gently boil off the carbon dioxide. When effervescence ceases, cool the basin slightly and remove the watch-glass, washing its under surface into the basin. Wash down the basin wall and then evaporate the contents to about 10 ml on a water bath. Add 0·5 g of boric acid and swirl to dissolve. Filter the contents through a paper-pulp pad into a 100-ml conical flask, washing the basin and finally the pad with hot water. About three 5-ml washes are required for the basin and a final wash for the pad. Drain the stem of the funnel into the flask. The final volume should be about 30 ml. If little or no carbon is present, the filtration may be omitted, the content of the basin being decanted into the flask and made up to 30 ml.

Add 5 ml of sodium molybdate solution and 5 ml of concentrated hydrochloric acid and heat to boiling. Add 1 to 2 drops of quinoline hydrochloride solution from a burette with swirling. Boil and add a further 2 drops. Continue in this manner, but increase the additions to 6 to 10 drops until 2 or 3 ml have been added, and then increase to 0·25-ml portions until a total of 5 ml of reagent are present. Swirling the flask during the additions and boiling the contents are necessary to obtain a coarse crystalline precipitate. Heat the flask on a water bath for 15 minutes and then cool it in running water.

Decant the supernatant liquid through a tight paper-pulp pad (about 0·5-cm thick). Wash the precipitate in the flask twice with 4-ml portions of dilute hydrochloric acid (1 + 9), decanting through the filter. Wash the precipitate from the flask on to the pad with cold water and continue to wash the flask until the washings are free from acid. Wash the funnel and precipitate with 5 to 10-ml portions of cold water, draining between washings, until the washings are free from acid. (Test the washings with litmus; about 6 washes are usually required.) Drain the funnel stem.

Transfer the precipitate and pad to the original flask with a clean glass rod and wash the funnel and rod with a jet of water into the flask until all traces of precipitate are removed. The volume should then be about 20 ml. Add 10 ml of 0·5 *N* sodium hydroxide to the flask and swirl to break up the pad and dissolve the precipitate completely. If necessary, stopper the flask and shake it. Wash down the flask and stopper, if used, with a little water.

Add 3 drops of mixed indicator solution and titrate with 0·5 *N* hydrochloric acid from a 10-ml microburette. The approach of the end-point is indicated by a colour change from violet to pale green. Continue to a very sharp change to pale yellow. Subtract the volume of acid used from the volume of 0·5 *N* sodium hydroxide added (10 ml).

1 ml of 0·5 *N* sodium hydroxide ≡ 0·5958 mg of phosphorus.

NOTE—

Although we have found negligible blank values with AnalaR reagents, it is advisable to check the reagents by carrying out a blank determination with 50 mg of sucrose as organic material.

RESULTS AND CONCLUSIONS

The results obtained by the method for the determination of phosphorus in a number of fluorinated compounds prepared during research are given in Table III. Full details of

duplicate analyses are given in order to show the range of sample sizes and amounts of phosphorus determined by this method.

TABLE III

ANALYSIS OF FLUORINATED COMPOUNDS

Compound	Sample weight, mg	Phosphorus calculated, mg	Phosphorus found, mg	Phosphorus calculated, %	Phosphorus found, %	Absolute error, %
$(C_3F_7{\cdot}CH_2{\cdot}O)_3PO$	57·22	2·75	2·78	4·81	4·87	+0·06
	49·40	2·38	2·39	—	4·84	+0·03
$(C_3F_7{\cdot}CH_2{\cdot}O)_2P(O)NH{\cdot}C_6H_4{\cdot}CH_3$	49·57	2·79	2·81	5·62	5·67	+0·05
	51·31	2·88	2·86	—	5·58	−0·04
$(C_3F_7{\cdot}CH_2{\cdot}O)_2P(O)NH{\cdot}C_6H_5$ ..	49·55	2·86	2·87	5·77	5·80	+0·03
	38·44	2·22	2·20	—	5·72	−0·05
$C_3F_7{\cdot}CH_2{\cdot}O{\cdot}P(O)(NH{\cdot}C_6H_4{\cdot}CH_3)_2$	23·51	1·59	1·58	6·76	6·74	−0·02
	21·60	1·46	1·46	—	6·76	0·00
$CF_3{\cdot}CH_2{\cdot}O{\cdot}P(O)(NH{\cdot}C_6H_5)_2$ ..	18·98	1·78	1·75	9·38	9·24	−0·14
	18·49	1·73	1·71	—	9·24	−0·14
$(CF_3{\cdot}CH_2{\cdot}O)_2P(O)NH_2$	24·31	2·88	2·86	11·87	11·77	−0·01
	19·36	2·30	2·27	—	11·75	−0·12

Although the presence of fluorine in the molecule of an organic compound does not necessarily invalidate the method of Belcher and Godbert for the determination of phosphorus, our experience has shown that the method is not universally applicable to fluorinated materials. We do not know the reason for the difference in behaviour of the compounds under acid attack.

The recommended fusion with sodium peroxide has, however, proved to be a successful method for opening up all the compounds encountered so far. Points of interest in this method are as follows—

(*a*) The use of the micro bomb for semi-micro amounts of material. It will be seen that over 50 mg of material can be decomposed by using 1 g of sodium peroxide and 50 mg of potassium nitrate. When these amounts were used, no addition of sucrose was made. We have not been able to find any reference in the literature that gives an explanation of the need for the addition of sucrose in peroxide fusions. We are of the opinion that this reagent could be omitted from fusion mixtures, but have included the addition of sucrose in our recommended method as we have not yet investigated this point fully.

(*b*) The use of platinum apparatus for weighing the sodium peroxide and in leaching the residue from the bomb assists materially in eliminating the blank values caused by the pick-up of silica. It is interesting to note that easily measurable contamination by silica was found when the peroxide was weighed on a watch-glass.

In the presence of fluoride, the leaching of the residue from the bomb, acidification and evaporation must be carried out in platinum apparatus or very serious errors caused by high blank values will result. On the other hand, when platinum apparatus is used, the presence of fluorine assists in reducing the blank values by removing silica as silicon tetrafluoride during evaporation of the acidified leachings.

(*c*) Wilson's method for the determination of phosphate has proved, with modification, to be suitable for the determination of 1 to 3 mg of phosphorus.

(*d*) Results should come within $\pm$0·15 per cent. (absolute), and it will be noted that the majority of the results obtained on fluorinated materials are within $\pm$0·05 per cent.

(*e*) A single determination can be completed in about 2 hours.

(*f*) It is thought that arsenic would interfere, although we have not tested the method in the presence of arsenic. Silicon does not interfere if sufficient fluoride is present to remove silicon as the tetrafluoride or if the bomb leachings are evaporated to dryness twice after acidification with hydrochloric acid.

(*g*) The method is being applied on the micro scale (for the determination of 0·2 to 0·5 mg of phosphorus) with encouraging results.

We thank Mr. E. J. P. Fear and his colleagues for the provision of samples and for their interest in this work.

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An Automatic Apparatus for the Quantitative Micro-determination of Nitrogen

By F. E. Charlton

A detailed description is given of an automatic apparatus for the quantitative micro-determination of nitrogen. The method is similar to the micro-Dumas method, and gives equally good results, but has the advantages that time is saved per determination and that the apparatus does not require the individual attention of the operator for the whole time during which a determination is in progress.

The work was undertaken because the micro-Dumas method for nitrogen, although giving excellent results for certain compounds, is time-consuming and requires close supervision. It was not hoped to improve on the results obtainable by the standard method, but to reduce the time factor, although it was recognised that an automatic apparatus would rule out some errors arising from personal manipulation.

Experimental

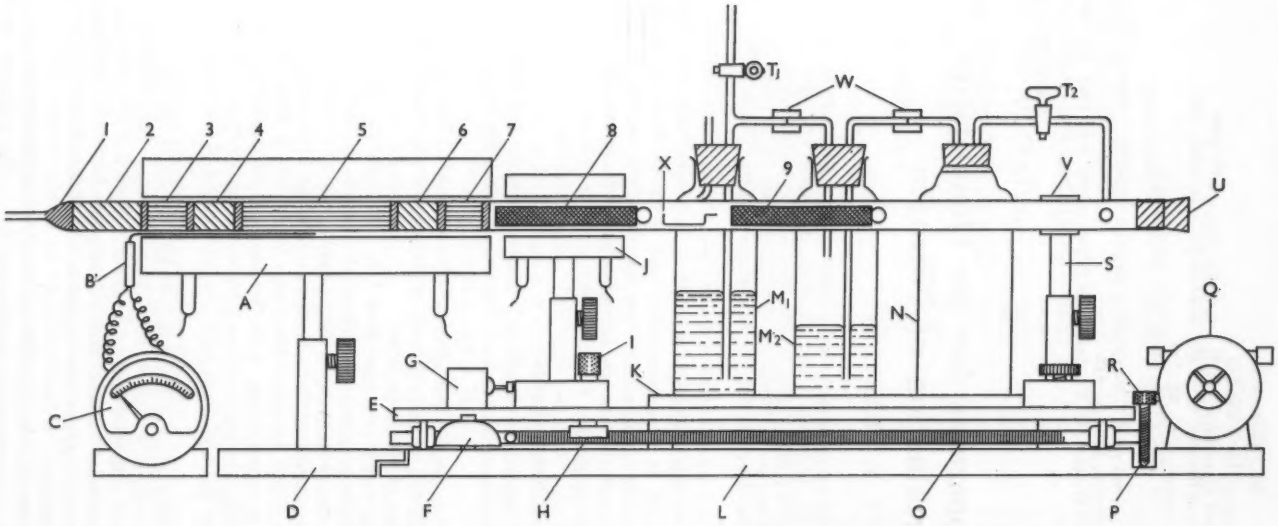
Fig. 1 shows the lay-out of the apparatus developed, the disposition and proportions of the filling materials, the lengths and temperatures of the heaters and the heights of the mercury columns.

The combustion tube conforms to the British Standard,[1] except that the side-arm is modified to simplify connection with the carbon dioxide generator train, which, to save bench space, is located behind the rest of the apparatus.

The carbon dioxide generator, a 1-pint vacuum flask,[2] N, will contain enough solid carbon dioxide[2,3,4] for a week, and the pressure is controlled by two bottles[4] containing columns of mercury, M_1 and M_2, 75 and 50 mm high, respectively, the one with the longer column serving to cushion pressure variations; there is carbon dioxide above the mercury in each bottle. The generator train is set in a wooden base, K, of which the end nearer the flask is removable to permit easy replenishing, and the components are connected by rubber tubing, which affords a flexibility desirable when disconnecting the flask. The ends of the delivery tubes into the flask are flat and flush with the under side of the rubber bung to permit air to be expelled freely.

The combustion tube, with its permanent filling lying within the main heater, remains *in situ* once it is filled, being clipped firmly to a support near the side-arm. This stable set-up is possible because the usual removable filling of finely powdered copper oxide is absent.[2,3,5] It is replaced by two copper oxide gauze rolls between which the sample boat[2,3,5] is positioned. The permanent filling, sections of reduced copper and copper oxide, differs from the normal filling for the micro-Dumas determination.

The temperature of the main heater, A, which is 200 mm long, is recorded by a chromel - alumel[6] thermocouple, B, connected to a temperature recorder,[6] C. The traverse of the automatic[2,5,6,7] movable heater is effected by means of a half-nut, H, operating on a worm



A	= main heater, length 200 mm, temperature 710°C	L	= wooden base
B	= thermocouple	M_1 and M_2	= glass bottles containing mercury, levels 75 and 50 mm
C	= temperature recorder	N	= 1-pint vacuum flask
D	= wooden base	O	= worm operating the half-nut
E	= steel platform	P	= driving cog for worm
F	= bell	Q	= electric motor
G	= universal micro switch	R	= friction drive
H	= half-nut drive for movable heater	S	= support for combustion tube
I	= quick release	T_1 and T_2	= glass taps
J	= movable heater, length 80 mm, temperature 900°C	U	= rubber bung
		V	= clip
K	= wooden base for carbon dioxide train	W	= rubber connections
		X	= platinum boat

Fig. 1. Automatic apparatus for the micro-determination of nitrogen. Filling of combustion tube: zone 1, 5 to 7 m of asbestos as a choking plug; zone 2, 75 mm of copper oxide; zone 3, 25 mm of copper; zone 4, 25 mm of copper oxide; zone 5, 90 mm of copper; zone 6, 25 mm of copper oxide; zone 7, 25 mm of copper; 8 and 9, 80 to 90-mm rolls of copper oxide gauze; the zones are separated by 2-mm plugs of asbestos

screw, O, driven by an electric motor, Q. The heaters and the motor are fed from a single[6] 5-amp. a.c. point. The movable heater, J, which is 80 mm long, having completed its traverse, is halted by a micro-switch, G, which rings a battery-operated bell, F. By means of a quick release device,[6] I, the movable heater may be placed anywhere along the combustion tube. The bases of the main heater, D, and the driving mechanism, L, are separate so that the main heater may be moved to inspect the filling for deterioration.

The micro-nitrometer will hold a maximum of 1·5 ml. It had been noticed that neither occasional large bubbles nor a slightly increased rate of flow affected the results obtained. When the main heater was left on,[8] no noticeable effect on the results was observed. So little air is introduced between determinations when the boat technique is used that a counter-current system, to protect the permanent filling, is considered an unnecessary complication. Experiments were conducted with the main heater at 710° C with carbon dioxide passing[9] at 2 or 3 bubbles per second instead of the conventional 2 bubbles per 3 seconds. Although more large bubbles left the mercury surface than usual, all had diminished to "micro-bubbles" before reaching the 1·1-ml graduation. The sample is adjusted to produce less than 1·1 ml of nitrogen.

In the conventional method sweeping out follows combustion. It was decided to sweep out the products of combustion as they formed with the main heater at 840° to 850° C to favour decomposition. Duplicate determinations on acetanilide gave 10·7 and 10·75 per cent. of nitrogen, which is too high, as the theoretical content is 10·4 per cent. Inspection of the filling showed more deterioration than usual with the main heater at 710° C. After carbon dioxide alone had passed for 10 to 12 hours, deterioration was marked. Fig. 2 shows the reactions put forward to explain the changes observed. Carbon monoxide and oxygen, which may result from dissociation of carbon dioxide, will recombine in region D, but carbon monoxide produced in region C will have no free oxygen with which to react and so may pass unchanged into the nitrometer. The small amount of water involved comes from the solid carbon dioxide for the carbon dioxide is not dried, as a drying train would not deal with the water formed from a sample. If the reactions are assumed to be materially correct, some carbon monoxide would pass into the nitrometer to be measured as nitrogen and high figures would result. In this respect therefore the theory fits the findings. With the main-heater temperature lowered again to 710° C, triplicate determinations on acetanilide gave 10·6, 10·5 and 10·55 per cent. of nitrogen, and these results all fall within the 0·30 per cent. deviation normally allowed. These figures are still slightly higher than the theoretical, so the length of the cool beak-end filling of copper oxide was increased[7] from 50 mm to 75 mm, so that more time was allowed for any carbon dioxide that may have dissociated[7,8] to re-associate. Triplicate determinations on acetanilide gave 10·4, 10·4 and 10·3 per cent. of nitrogen, and these results were satisfactory.

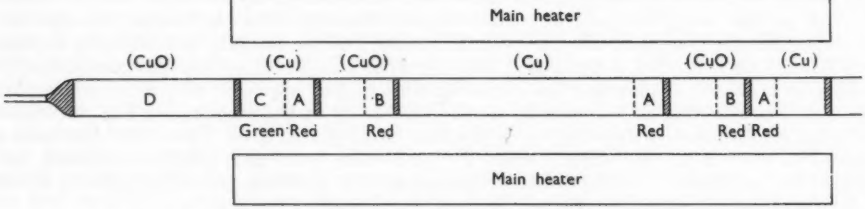


Fig. 2. Reactions in the combustion tube. The original conditions of filling are shown in brackets above the tube. The subsequent reactions are as follows—

Region A: $2Cu + H_2O \rightarrow H_2 + Cu_2O$ (red) (hot junctions in copper regions)
Region B: $2CuO + H_2 \rightarrow Cu_2O + H_2O$ (hot junctions in copper oxide regions)
Region C: $2Cu + 4CO_2 + H_2 \rightarrow 3CO + CuCO_3.Cu(OH)_2$ (green)
Region D: cool, as it is not enclosed by the heater; there is little reaction and any carbon monoxide passes through unchanged

A further modification made was that the sample was sandwiched between two layers of copper oxide.[3] Solids having high melting-points would mix more intimately when they melted and sank into the lower layer.

Several samples of research compounds when analysed gave good results with the

modified conditions. Liquids were analysed satisfactorily when the open tip of the sample tube was dipped into a quantity of finely powdered copper oxide in the front end of the platinum boat.

It was found necessary to change the potassium hydroxide solution in the nitrometer after 8 or 10 determinations.

Use of the apparatus

The flask, N, filled with finely powdered solid carbon dioxide is connected to the apparatus with the tap, T_2, leading to the combustion tube kept closed, and carbon dioxide is allowed to bubble through the mercury, for a minimum of 4 to 5 hours, preferably overnight. The remaining solid carbon dioxide is free from air and is adequate for 4 to 5 days.

A newly filled combustion tube is stabilised by raising the main heater to the operating temperature (710° C) and cooling to room temperature three times during the passage of a slow stream of carbon dioxide. At the end of this procedure, which serves to free the permanent filling from air, the apparatus is ready for use and no further stabilising is necessary unless the apparatus is idle for a long period. Subsequently, when the solid carbon dioxide is being replenished, the flask is disconnected with the apparatus cold and the connecting tap is closed, the beak end of the combustion tube being capped so that the permanent filling is maintained in an atmosphere of carbon dioxide under pressure. After the conditions described in the previous paragraph have been fulfilled, a determination can be carried out.

With the main heater at 710° C, the sample for analysis is positioned as follows. The connecting tap is opened fully and the beak end of the combustion tube is capped. The tap is then closed and the rubber bung, U, is removed from the combustion tube. A copper oxide gauze roll, 8, is inserted to touch the permanent filling, and the platinum boat, X, containing the sample sandwiched in finely powdered copper oxide, is introduced nearly to touch the gauze. The other gauze roll,[3] 9, is positioned nearly touching the sample boat. The connecting tap is opened fully so that the rapid backflow of carbon dioxide expels air that may have diffused into the combustion tube and bung U is replaced as the mercury surfaces in the delivery tube and the first mercury bottle become level. The nitrometer is connected to the combustion tube, after the potassium hydroxide solution has been drained from the mercury, and a 3-minute sweep-out with carbon dioxide is made with all taps fully open. With the nitrometer set for gas collection, and the connecting tap with the generator fully open, the rate of flow is set at 2 or 3 bubbles per second by means of the nitrometer side-arm tap. The movable heater, with the sample boat just under the end nearer the main heater, is raised[10] to 900° C in 10 minutes and set in motion. The traverse of 8·5 cm takes 11 minutes, by which time the bubbles, which increase in size 4 to 5 minutes after heating commences, diminish considerably in size. The rate of burning[11] is vital. It will be effected by both the traverse time and the rate of flow of carbon dioxide. With simple compounds such as acetanilide, it was found possible to reduce the traverse time to 5 minutes, the rate of flow being unchanged. Twenty-seven minutes after the heating has started the movable heater is switched off, the bubbles having become minute, but bubbling is allowed to continue for a further 5 minutes. The nitrometer is disconnected, the combustion tube is capped, and the connecting polythene tubing to the reservoir of potassium hydroxide solution is constricted and tapped to break down foam at the meniscus. The nitrometer is set aside for 15 minutes and the nitrogen value is calculated after 1 per cent. has been subtracted to correct for the error caused by potassium hydroxide solution adhering to the walls of the nitrometer. If the drainage time exceeds 30 minutes, greater accuracy is obtained if no correction is made.

Results

The formulae of some of the research compounds analysed by this method, which contained from 3·6 to 66·6 per cent. of nitrogen, and the results obtained, are shown in Table I, together with the results for four standard compounds.

Scope and validity of results

The method does not appear so far to have any marked advantages over the micro-Dumas method, with regard to the type of compound that may be analysed.

A great advantage is in the time saved, for, if two nitrometers and two sets of copper oxide gauze rolls are used, a determination can be made every 35 minutes. Previously only

four determinations per day were possible, because, although the increased rate of flow may be applied equally well to the old apparatus, the attention of the operator was required for the manipulation of the movable heater, usually a bunsen burner.

Some of the research compounds analysed are known and details are published in the literature. Their purity was checked by physical characteristics before submission for analysis. Other compounds, although new, have shown predictable characteristics, so there is no reason to assume anything odd about their behaviour. In addition, the compounds analysed included such micro-analytical standards, used for check purposes, as acetanilide, 8-hydroxyquinoline, phenylthiourea and S-benzylthiouronium chloride.

TABLE I

DETERMINATION OF NITROGEN IN VARIOUS COMPOUNDS

Compound	Nitrogen found, %	Nitrogen calculated, %	Compound	Nitrogen found, %	Nitrogen calculated, %
Micro-analytical standards—					
Phenylthiourea ..	18·3	18·4	Acetanilide	10·4*	10·4
S-Benzylthiouronium chloride	13·9	13·8	8-Hydroxyquinoline ..	9·6	9·65
Research compounds—					
$C_{14}H_{23}N$	6·7	6·8	$C_{10}H_{22}N_2$	16·5	16·45
$C_2H_4N_4$	66·5	66·6	$C_2H_5O_2N$	18·9	18·7
CH_4ON_2	46·8	46·7	$C_7H_7O_2N_3$	23·2	23·2
$C_{12}H_6O_4N_6$	22·2	22·2	$C_{22}H_{19}O_{21}N_{13}$	21·3	21·3
$C_9H_{10}NBr$	6·8	7·0	CH_4N_2S	37·1	36·8
$C_8H_{12}N_2Cl$	22·4	22·6	$C_8H_{11}O_2NS$	7·65	7·6
$C_8H_6ONF_3$	7·7	7·4	$C_{10}H_{16}O_2NP$	6·7	6·6
$C_{16}H_{20}O_4N_2S_2$	7·1	7·0	$C_8H_{11}O_2N_2I$	9·55	9·5
$C_{10}H_{25}ON_3P$	11·7	11·9	$C_{11}H_{12}O_6N_2Cl$	13·1	13·2
$C_{20}H_{25}O_7N_4Br$	11·0	10·9	$C_{16}H_{18}O_8NS_2Cl$ ..	4·1	3·9
$C_{15}H_{22}O_2NS_2Br$ ..	3·6	3·6	$C_{10}H_{17}O_3N_2SP$ ..	10·6	10·8

* Average of 11 determinations.

DISCUSSION

Kirsten[2] has remarked that the quantitative micro-determination of nitrogen along the lines of the Dumas method appears to be very simple, but actually the factors involved are many and complex. Many of the possible sources of error in this method appear to have been eliminated.[12] It can be argued that the errors may still exist but are compensatory. It is most unlikely in view of the range of compounds analysed that this should be so.

Use of the boat technique largely rules out errors due to air occluded in the temporary filling.[11,12,13]

Heater temperatures have been the subject of much discussion. According to Kirsten,[2] rapid auto-decomposition of methane appears to occur at 700° to 800° C. Two conditions arise at lower temperatures (*a*) high results from methane produced by incomplete combustion and (*b*) low results from nitrogenous charcoal formation. Kirsten does not consider condition (*b*) to be so prevalent as commonly imagined. Higher temperatures favour retention of oxides of nitrogen as a result of the formation of oxygen from copper oxide.[14]

Kirsten[8] found 750° to 800° C adequate for primary heating of the sample mixed with copper oxide in a quartz capsule. Brancone and Fulmor[10] obtained good results with the movable heater at 900° C. It would appear that, with movable-heater temperatures ranging from 750° to 900° C, nitrogenous charcoal is not formed, and moreover that the sample in this region volatilises before the temperature becomes high enough to favour retention of oxides of nitrogen. Thereafter the volatilised sample is driven before the movable heater and is never subject to the full 900° C.

With the main heater at 840° to 850° C, high results were obtained and these were reduced when the temperature was lowered to 710° C. The high results at the higher temperature were unlikely to be due to either incomplete combustion resulting from the formation of methane or retention of oxides of nitrogen, since at the lower temperature less retention of nitrogen oxides and less complete combustion, both of which would produce higher results, would be expected.

Dissociation of carbon dioxide[8] may account for high values found at 840° to 850° C with a rate of flow of 2 or 3 bubbles per second, but would not explain the changes observed

in region C (see Fig. 2). It could, however, account for those results found to be slightly higher than theoretical with the shorter cool beak-end filling of copper oxide.

Apparently by this technique the temperature of the movable heater is not vital, provided it is adequate for combustion, but the temperature of the main heater is important. With this 710° C seems to provide for most contingencies, falling as it does within the suitable range, 700° to 800° C, mentioned by others.[2,12]

Nitric oxide[15,16] may cause errors. Zimmermann[16] overcame this by leading the gases twice over parts of the tube filling before they entered the hot central zone of copper. In the above-mentioned method the gases pass over a copper oxide gauze roll, copper (wire-form) and copper oxide (wire form) before entering the hot central copper zone.

Finally, mention is made of diffusion errors[2,8,13,17,18,19] arising from connections and bungs. Polythene has been found suitable for connections not subject to temperatures much in excess of normal room temperatures, but it is not agreed that diffusion occurs through bungs. Errors from bungs have been observed, particularly during carbon and hydrogen determinations, but these were undoubtedly due to channelling between worn bung surfaces and glass walls. This could apply equally well to nitrogen determinations.

I thank the Chief Scientist, Ministry of Supply, for permission to publish this paper.

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Notes

THE POLAROGRAPHIC DETERMINATION OF BORIC ACID BY MEANS OF SODIUM NITRITE

Lewis[1] has shown recently that boric acid, in the presence of sodium sulphite and mannitol, gives rise to a polarographic wave that may be used for its determination. This Note describes the results obtained when sodium nitrite is substituted for sodium sulphite in order to increase the sensitivity of the method.

Experimental

All solutions were examined at 25° ± 0·1° C and the dropping-mercury electrode had a characteristic of 2·05 mg $^{2/3}$ sec.$^{-1/2}$ in 0·48 *M* mannitol and 0·2 *M* sodium nitrite at an applied potential of −0·78 volt against a saturated-calomel electrode (S.C.E.).

Preliminary experiments showed that boric acid, in the presence of mannitol and sodium nitrite, gave rise to two polarographic waves, the first at −0·64 volt and the second at −1·21 volts against the S.C.E. The optimum base-electrolyte composition for maximum sensitivity was found to be 0·48 *M* in mannitol and 0·2 *M* in sodium nitrite. It was noted that the diffusion currents increased during the first 10 minutes after the solutions were prepared and then decreased slowly. The height of the first wave and the total height of both waves, measured 10 minutes after the solutions had been prepared, were found to be directly proportional to the boron concentration over the range 2 to 20 μg per ml in the final solution. When a Cambridge pen-recording

polarograph was used, the wave due to 1 μg of boron per ml was detectable, but not measurable.

The diffusion-current constant of the total wave was 1·52 (μA per mg $^{2/3}$ sec.$^{-1/2}$ per millimole), and that of the first wave only, 0·814.

The wave height due to 20 μg of boron per ml in the mannitol - nitrite base electrolyte was nearly forty times greater than that obtained with a mannitol - sulphite base. With 200 μg of boron per ml, however, the increase in sensitivity was reduced to a factor of about three.

Discussion of method

Lewis reported the following relationship between the concentrations of boron, c, hydrogen ion, H^+, and the diffusion current, i_d, of the first wave—

$$\frac{[i_d]^2}{[c]\ [H^+]^2} = \text{a constant.}$$

He attributed the first wave to the reduction of bisulphite ion (formed by reaction of mannito-boric acid with sodium sulphite) and accepted the mechanism for this reduction suggested by Kolthoff and Miller,[2] *viz.*—

$$HSO_3^- + 2\,H^+ + e^- \longrightarrow HSO_2 + H_2O.$$

This, however, is not consistent with the relationship shown above and, in the presence of mannitol, the electrode reaction is more probably—

$$2\,HSO_3^- + 2\,H^+ + 2\,e^- \longrightarrow S_2O_4^{2-} + 2\,H_2O.$$

The reduction mechanism of nitrous acid is not known with certainty, but our findings confirm those of Keilin and Otvos,[3] who reported that the diffusion current declined with time, owing to the spontaneous decomposition of the nitrous acid. They therefore recommended that the nitrite and acid solutions should be de-oxygenated separately before the mixing and polarographic determination. This procedure has been adopted in the method proposed for the determination of down to 2 μg of boron, as boric acid per ml, in the final solution. Lewis has suggested suitable methods of preparing boric acid solutions for polarographic determination, from various types of sample.

Method

Reagents—

Mannitol, 0·8 M—Dissolve 14·6 g of mannitol in water and dilute to 100 ml with water.

Sodium nitrite, 1·0 M—Dissolve 7·0 g of sodium nitrite in 100 ml of water.

Procedure—

Place 3·0 ml of 0·8 *M* mannitol solution and 1·0 ml of 1·0 *M* sodium nitrite solution in a polarographic cell and de-oxygenate. Add 1·0 ml of the de-oxygenated sample solution, mix with a stream of nitrogen for about 20 seconds and set aside for 10 minutes with air excluded from above the solution. Record a polarogram over the range 0 to −1·2 volts, using a mercury pool as anode. Measure the height of the step that occurs at a half-wave potential of about −0·70 volt, using the intersection method of step-height measurement. Read off the amount of boric acid in the sample from a calibration graph prepared by using solutions containing known amounts of boric acid in place of the sample solution and proceeding exactly as described above.

Conclusions

Sodium nitrite can be used instead of sodium sulphite in the method proposed by Lewis for the polarographic determination of boron. The disadvantage of the substitution is that the nitrite base electrolyte and sample require separate de-oxygenation before the polarographic determination. However, there is a linear relationship between the diffusion current and the boron concentration, and the method is more sensitive by a factor of three at the 200 μg of boron per ml level and by a factor of about forty at the 20 μg of boron per ml level.

I am indebted to Mrs. S. Brown for carrying out much of the polarography reported in this paper.

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A FURTHER STUDY OF THE DETERMINATION OF THIAMINE WITH 6-AMINOTHYMOL

An extended investigation of the method of determination of thiamine based on the measurement of the colour produced with diazotised 6-aminothymol[1] has revealed that diazotisation is not necessarily involved in the thiamine reaction and that a similar colour is produced by direct treatment of the vitamin with an alkaline solution of 6-aminothymol. Colour development is not as rapid as with the original diazo reagent, but is more quantitative, and interference from phenols is greatly reduced.

The object of our investigation was to make a comparison of the development and stability of the colours obtained with and without the addition of nitrite, and to examine further the extent of interference from specific substances employed in the extraction procedures or added as preservatives. A modified method of extraction also described permits the application of the 6-aminothymol method to materials containing creta praeparata.

Experimental

Colour development—

In experiments made to compare the rates of colour development in the original and modified procedures, optical densities were measured at 20° C in a Spekker absorptiometer. The curves obtained by plotting optical densities against time are shown in Fig. 1. These indicate that, although the reaction is slower when the diazotisation step is omitted, the colour so formed is no less stable than when nitrite is present. A similar series of tests showed that, within the range 15° to 25° C, light and temperature have a negligible effect on the colour.

Fig. 2 shows curves obtained by the two methods with amounts of thiamine up to 120 μg. The final volume of the solutions was 10 ml in each experiment. Curve B represents measurements made after 15 minutes' development and shows that Beer's law is obeyed over the entire range when nitrite is omitted. It is concluded, therefore, that the described modification extends the useful range of the method and yields lower reagent blanks than the diazo procedure. Further, the alkaline reagent solution was found to remain active for at least 30 minutes after preparation.

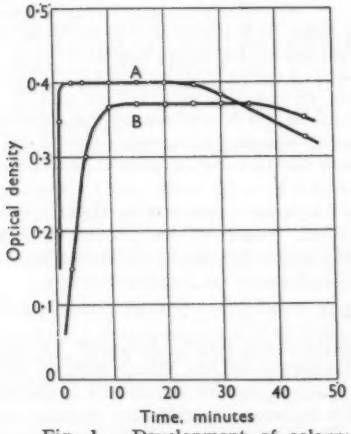

Fig. 1. Development of colour: curve A, with nitrite; curve B, without nitrite

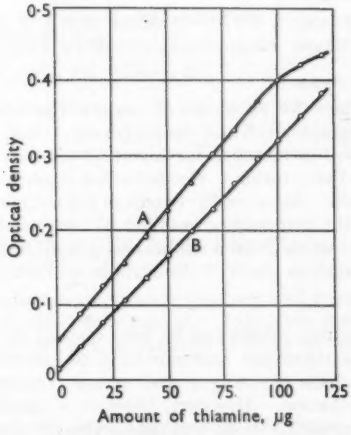

Fig. 2. Standard curves: curve A, with nitrite; curve B, without nitrite

Interference—

In general, the modified method was found to be subject to interference from the same classes of materials as formerly described, the most serious errors arising from suppression of colour development by the more powerful oxidising and reducing substances. The reagent produces a range of colours with phenolic compounds, but these are different from and less intense than those formed with diazotised 6-aminothymol. The phenols as a class do not cause a serious interference, and phenol, cresol, chlorocresol, chloroxylenol and the hydroxybenzoic acid derivatives

yield no measurable colour with the reagent when they occur at the levels usually employed for preservative purposes. The commonly used protein precipitants, such as lead or zinc salts or trichloroacetic acid, have little effect on the colour. High concentrations of alkali-metal salts have little influence on either the rate of colour development or its intensity, but the thiamine reaction is slow and incomplete in the presence of substantial amounts of ammonium salts.

EXTRACTION PROCEDURE—

Ridyard[2] pointed out that thiamine is extracted with difficulty from flour-enrichment materials containing creta praeparata and showed that solution of the sample in strong acid was essential for adequate recovery of the vitamin from mixtures of 1 part of Master Mix to 14 parts of creta praeparata. In this laboratory Ridyard's method has not always given satisfactory recoveries. Further, extraction of such mixtures with acid presents difficulties in the 6-aminothymol procedure, owing to subsequent precipitation of calcium salts; for these reasons, an alternative method of extraction was sought. The results collected in Table I show that extraction with saturated potassium chloride solution yields figures satisfactorily close to the expected thiamine content. The Table also shows that, when calcium carbonate is present in the form of precipitated chalk, adsorption of the vitamin does not occur and the extraction can be satisfactorily accomplished with water.

TABLE I

EXTRACTION OF THIAMINE IN THE PRESENCE OF CALCIUM CARBONATE

Each sample was prepared to contain 0·05 per cent. of thiamine

Base of mix	Extraction method	Assay method	Thiamine found, %
Precipitated chalk	Water, 10 minutes	Thiochrome	0·050, 0·051
	Water, 10 minutes	6-Aminothymol	0·050, 0·049
	Water, 5 hours	6-Aminothymol	0·051
	Water, 12 hours	6-Aminothymol	0·048
Creta praeparata	Acid	Thiochrome	0·046, 0·042
	Saturated potassium chloride	Thiochrome	0·050, 0·050
	Water	6-Aminothymol	0·035
	Saturated potassium chloride	6-Aminothymol	0·049

It is worthy of note that the above tests relating to creta praeparata were carried out 6 months or more after preparation of the mixtures. The results confirm Ridyard's conclusions about the stability of thiamine in the presence of creta praeparata and that reports of vitamin destruction in such mixtures may be attributed to the poor recovery of added thiamine when the usual methods of extraction are employed.

METHOD

The method of determination[1] is unchanged in principle, but the amendments indicated below are necessary.

REAGENTS—

Alkaline 6-aminothymol solution—Dissolve 50 mg of 6-aminothymol hydrochloride in 50 ml of 0·1 *N* hydrochloric acid and dilute with water to 100 ml. For use, treat 5·0 ml of this solution with 5·0 ml of 20 per cent. sodium hydroxide solution and dilute with water to 20 ml. The alkaline reagent may be used up to 30 minutes after preparation.

Potassium chloride solution—Prepare a stock solution by adding 250 g of reagent grade potassium chloride to 500 ml of distilled water, and decant from undissolved solid as required.

EXTRACTION OF SAMPLES—

(*a*) *Samples containing metals, metal salts or other substances reacting with thiamine in acid solution*—To an accurately weighed portion of the material add sufficient pure calcium carbonate in the form of precipitated chalk to neutralise subsequently any free acid present. Extract with water by shaking for 10 minutes, filter and dilute the filtrate so that the final solution contains 20 to 40 μg of thiamine per ml.

(*b*) *Samples containing creta praeparata*—Extract a weighed portion of the sample with a measured volume of saturated potassium chloride solution, shaking the mixture vigorously for at least 10 minutes. Filter and dilute as in (*a*).

PROCEDURE—

As formerly described, but with 15 minutes allowed for maximum colour development.

RESULTS

Table II shows the results obtained by the original diazo method and by the proposed modification when applied to the same extracts from a series of 12 samples of Master Mix. Agreement is shown to be excellent and the small differences are within the limits of experimental error.

TABLE II

RESULTS OBTAINED BY THE TWO METHODS

Thiamine found by original method, %	Thiamine found by modified method, %	Difference, %
0·74	0·73	−0·01
0·73	0·73	0
0·75	0·75	0
0·72	0·72	0
0·74	0·75	+0·01
0·77	0·76	−0·01
0·76	0·75	−0·01
0·73	0·72	−0·01
0·73	0·72	−0·01
0·74	0·74	0
0·78	0·78	0
0·78	0·79	+0·01

We thank the Directors of Novadel Ltd. for permission to publish this Note.

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NOVADEL LTD.
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February 27th, 1957

THE USE OF ALKALI HYDROXIDES FOR THE SEPARATION OF THE COPPER AND ARSENIC GROUPS

IT has been recommended[1,2] that tin^{II} should be oxidised before precipitation as sulphide if alkali hydroxides are to be used for the separation of groups 2A and 2B, although the necessity for this has been disputed.[3] Hydrogen peroxide, bromine water and sulphur dioxide have all been recommended, and criticised on the grounds of their effect on other ions that may be present. An oxidant that is free from these objections is mercuric chloride, but its use entails the previous detection of the tin^{II}. If tin^{II} is present, there should not be any $mercury^{II}$ co-existent with it, and, if it is absent, the need to oxidise it does not arise; there is no risk of failing to detect any $mercury^{II}$ originally present. All that is necessary is to apply the cacotheline spot-test for tin^{II} after the separation of group 1 and, if tin^{II} is found, to oxidise it with mercuric chloride. The mercurous chloride can be separated and the group 2 precipitation made in the usual way. The mercuric sulphide will be found in both group 2A and 2B, and is ignored in the report. We have found that the only common ion that interferes in the cacotheline test is thiosulphate, and then only if it is present in relatively large amount. In any event, the thiosulphate would almost certainly be decomposed before the cacotheline test could be applied.

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February 21st, 1957

THE APPLICATION OF DIPHENYLAMINE AND RELATED COMPOUNDS TO SPOT-TESTS FOR NITRATE AND NITRAMINE EXPLOSIVES

DIPHENYLAMINE in concentrated sulphuric acid solution is commonly given in text-books as a spot-test reagent for nitrates and its use has been suggested for nitramines.[1] Various workers have found that the concentration of the sulphuric acid used in testing for nitrate is important if the results are to be reproducible,[2,3,4] and we have found that the degree of dilution is also important when diphenylamine and other reagents are applied to test for nitramines. The reagents used were the solutions in sulphuric acid of diphenylamine, diphenylbenzidine, nitrodiphenylamine and brucine. The nitramines examined were RDX (*cyclo*trimethylenetrinitramine), HMX (*cyclo*-tetramethylenetetranitramine), picrite (nitroguanidine) and tetryl (2:4:6-trinitrophenylmethyl-nitramine); two nitrate explosives, PETN (pentaerythritol tetranitrate) and urea nitrate, were also used for comparison. The method of test used was to place a few dry crystals of the substance under examination on a white spot-plate and add one drop of the reagent, the colour developed at various time intervals being noted. The tests were carried out a number of times by each of several operators to ensure their reproducibility.

Both the nitrates and the nitramines gave unsatisfactory results with diphenylamine when cold sulphuric acid in concentrations above 90 per cent. was used in making up the reagent. The most satisfactory results were obtained when 80 per cent. sulphuric acid was used and 5 minutes were allowed to elapse for the development of the colours. For all the nitramines tested the colour produced was blue, as for the nitrates. With the concentrated acid a blue colour developed only slowly, sometimes not for 60 minutes, and it was apparent that in these circumstances water had to be absorbed from the atmosphere.

If the reagent was warmed before it was applied, the rate of development of colour was accelerated and from this a means of distinguishing between RDX and HMX was developed. In these tests 88 per cent. sulphuric acid was used in making up the reagent; this gives a strong blue colour after 1 minute with RDX in the cold, but no colour with HMX until more than 5 minutes have elapsed. When the reagent is used warm, the HMX reacts as quickly as RDX.

Diphenylbenzidine and nitrodiphenylamine reagents reacted similarly to diphenylamine, but diphenylbenzidine gave more reproducible results and there is a clear advantage in using it. The use of brucine reagent was also investigated and it was found to give an orange colour with both the nitrates and the nitramines. In this instance it was found important to use the brucine dissolved in concentrated sulphuric acid, as dilution led to fugitive coloration.

It is concluded that all the reagents examined, diphenylamine, nitrodiphenylamine, diphenylbenzidine and brucine, will detect nitramines as well as nitrates, but can neither be used to distinguish between them nor to indicate individual compounds; that for successful use of the reagents the concentration of the sulphuric acid is important; and that warming increases the sensitivity of the test. By using diphenylamine it has been found possible to distinguish between RDX and HMX, because of their different rates of development of colour in the cold, and it is recommended that, when the presence of HMX is suspected, the reagent should be warmed to accelerate the reaction.

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ARMAMENT RESEARCH AND DEVELOPMENT ESTABLISHMENT
FORT HALSTEAD
KENT

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February 11th, 1957

Book Reviews

THE DETERMINATION OF TOXIC SUBSTANCES IN AIR: A MANUAL OF I.C.I. PRACTICE. Edited by N. STRAFFORD, C. R. N. STROUTS and W. V. STUBBINGS. Pp. xxviii + 226. Cambridge; W. Heffer & Sons Ltd. 1956. Price 35s.

This volume is a manual of methods used by Imperial Chemical Industries' personnel for the assessment of toxic concentrations in the air of their establishments, and is compiled by the Company's Analytical Chemists' Committee.

The publication may be regarded as a valuable contribution to the prevention of industrial poisoning. Incorporated in the volume are the methods suggested in the twelve D.S.I.R. pamphlets on the detection of toxic gases, but the main work is a compilation of detailed methods of analysis of some forty-nine toxic substances (commonly encountered in industry) that have been approved and have stood the test of experience under various working conditions.

The first part of the book deals with the physical side of the collection of samples, *i.e.*, the apparatus used for the measurement of air volume, the methods and apparatus used for the removal of the toxic substances and the mechanical apparatus for producing air flow. This includes a description of the D.S.I.R. pump, aspirator and vacuum procedures, flowmeters and absorption vessels, including various bubblers and impingers. Methods for the sampling of particulates are also given. The subsequent chapters are concerned with the detailed techniques for absorption and assessment of the commonly occurring toxic substances. Two methods of testing are given for each substance where possible. The first comprises a procedure that calls for skill in chemical manipulation and the use of a laboratory set-up. The second type of test advocated may be carried out without special skill and with relatively simple apparatus. The more elaborate tests are for use when precision is required, *e.g.*, in research and for legal purposes. The simpler procedures are advocated for on-the-spot factory working, *i.e.*, for determining whether an area is safe for employees. The lay-out of the various techniques given is very clear, details being given of the sampling procedure, the analytical method and the subsequent calculation. The book also contains a very useful Table giving the levels of concentrations of the various substances mentioned likely to produce (*a*) severe toxic effects and (*b*) illness if prolonged exposure occurs and also giving limits for satisfactory working.

It can be seen from this description that the book covers a wide field and should be of some value to all practising analysts and particularly those concerned with atmospheric pollution of any kind.

It must be stated as an opinion that the book is doubtless a good survey of the field and that the choice of methods is judicious. Research workers, however, might find the information given about the methods of determination rather inadequate. There are many pitfalls encountered during the assessment of abnormal substances in the atmosphere, not the least of which concerns the trapping of the sample. Each problem must be taken on its merits. The book tends to suggest that a standardised procedure can be followed, and experience in this field shows this to be a false assumption.

R. F. MILTON

CAROTENE: ITS DETERMINATION IN BIOLOGICAL MATERIALS. By V. H. BOOTH. Pp. viii + 119. Cambridge: W. Heffer & Sons Ltd. 1957. Price 18s.

Dr. Vernon Booth has taken a leading part in establishing satisfactory analytical procedures for carotenoids in products such as dried grass. Reports of collaborative work (Analytical Methods Committee, *Analyst*, **1950**, **75**, 568; **1952**, **77**, 171) showed that the methods he used were trustworthy and the results reproducible. The present monograph, which has been published under the auspices of the Society for Analytical Chemistry, summarises his very considerable experience. Few analysts have comparable experience in this field and nobody else could have written *this* book. It is refreshing to read a work on analytical chemistry in which the personal touch has not been revised away.

In explaining the book's arrangement the author writes as follows—

> "The diversion on spectrophotometry is treated with elementary detail because prospective carotenologists are often biologists, of whom some may perhaps have difficulty in finding the essential facts of colorimetry. The main feature of the book, the Working Directions, is divided into directions for carrying out the general method and descriptions of modifications for extracting particular materials. . . . The main theme, the working directions, may seem to be buried in a mass of incidentals. Therefore, in order to assist an enquirer to find them, the directions for the general method are printed on tinted paper."

The thoroughness of the work is illustrated by instructions for grinding samples in "heavy gauge 50 ml squat beakers" with pestles made from glass rods. "The shoulders must be sloping; if a moat is formed where the ball joins the stem, small particles of grist may lodge in it and escape extraction. The handle of each pestle is a cork bored part-way through. Medium fine quartz powder, approximately 80 mesh is much better than sand as a grinding agent. It is sharper and non-rolling and each particle after it is crushed presents new sharp edges to the grist instead of rounded ones."

When it is necessary to increase the rate of flow of liquid through a chromatographic column, Dr. Booth applies suction, but controls it with a foot pedal; when gas pressure is applied to the liquid over a chromatographic column, use is made of a specially designed wall fixture with a 4-kg weight.

Dr. Booth has a chapter on speed in routine assays. "The more obvious points, for instance that reagents must be in adequate supply, are not discussed," but the point is made that "in weighing a series of samples into beakers, the ideal is that every empty beaker shall have the same weight as the previous one plus its sample, for this demands the least adjustment of weights." "Solvent bottles must be of handy dimensions with stoppers of a type that suits the stopper drill of the laboratory. Bottles for commonly-used solvents—petrol, acetone, etc.—should not be uniform in shape and colour. Different shapes, coloured labels or decorative treatment help to avoid mistakes."

As a guide to the determination of carotene, this little book is the work of an expert, who in a chapter on alternative methods deals fairly with the literature. There is about the book, however, a special flavour that the experienced analyst will enjoy: "A blendor with a leaky gland must not be used"; "Freeze the margarine to facilitate weighing." Dr. Booth delights in good manipulation and clearly desires that the reader, too, shall be an impeccable craftsman.

R. A. MORTON

THE ANALYSIS OF MINERALS AND ORES OF THE RARER ELEMENTS. By the late W. R. SCHOELLER, Ph.D., F.R.I.C., and A. R. POWELL, F.I.M., F.R.I.C., F.R.S. Pp. xvi + 408. Third Edition. London: Charles Griffin & Co. Ltd. 1955. Price 60s.

All chemists concerned with the analysis of minerals and ores containing the rarer elements will welcome this new edition of what has become, in many laboratories, a standard book of reference. Since the second edition of this book appeared in 1940, the "rarer" metals have attained a new importance and considerable progress has been made in the study of their analytical chemistry. Mr. Powell is to be complimented on the highly successful way in which he has brought his book up to date, more particularly so since he lacked the help of his co-author, Dr. W. R. Schoeller, who died in 1947. The most noteworthy change in this edition is the considerable simplification of the author's method of presenting a vast amount of detailed information. The previous edition made use of a complicated system of cross reference, almost certain to confuse the reader. This has disappeared from the present edition, in which large sections of the book have been completely re-written, with the result that it is now easy both to read and to use as a reference book. This extensive re-writing is particularly true of the chapters on platinum metals, uranium, rhenium and scandium. Drawing on his considerable experience of platinum-metal analysis, Mr. Powell gives new procedures for the treatment of platinum-metal mixtures; for example, he describes a method for the separation of platinum, palladium and rhodium from iridium by precipitation with hypophosphorous acid in the presence of mercuric chloride. In this chapter and in others it is noteworthy that Mr. Powell gives not merely a list of analytical methods but expresses quite clearly, with reasons, which is the best method for a particular application in the light of the experience of himself and his colleagues. Throughout the book classical procedures have been revised, and in addition many applications of the more recently developed analytical techniques are described. These include the use of solvent-extraction methods for uranium, scandium and gallium; chromatographic methods for uranium, thorium, niobium and tantalum; polarographic methods for uranium, europium and ytterbium; spectrophotometric methods for the rare earths and the platinum metals. There are, of course, some omissions—no mention is made of the recently described colorimetric methods for niobium and tantalum and the much used fluorimetric method for the determination of uranium, but in attempting to cover such a wide field it is almost inevitable that something should be left out. Most inorganic analysts will find this book of invaluable help and senior students by reading it will learn a great deal of the chemistry of the rarer metals not always given in textbooks of inorganic chemistry. R. A. WELLS

SEMIMICRO QUALITATIVE ORGANIC ANALYSIS: THE SYSTEMATIC IDENTIFICATION OF ORGANIC COMPOUNDS. By NICHOLAS D. CHERONIS AND JOHN B. ENTRIKIN. Second Edition. Pp. xiv + 774. New York and London: Interscience Publishers Inc. 1957. Price $9.00; 72s.

The second edition of this book, well known to most workers in the field of organic analysis, bears slight resemblance to the original edition. It has been completely re-written, re-organised and expanded by some 250 pages.

In the 10 years that have elapsed since the appearance of the first edition, the semimicro method has become more and more recognised as the principal rather than the subsidiary method of chemical analysis, whether it be in the teaching of chemistry in universities and colleges or in its applications in industrial laboratories. The considerable impetus that has been given to the development of new methods and techniques of qualitative organic analysis fully justifies the appearance of this second, completely revised, edition.

The book is divided into four main parts. Part I deals with the techniques of organic analysis; Part II describes the procedures to be followed in the tentative identification of an unknown compound; Part III details the procedures for the final characterisation of the organic substances. Part IV contains almost 200 pages of tables listing the physical constants of organic compounds and their most useful derivatives.

Many new features have been introduced, among which may be mentioned the section on the use of infra-red spectra for the detection of specific groups and of Davidson's acid - base indicators as an aid to the classification of unknown compounds, the inclusion of a number of new reagents for the detection of functional groups, new separation procedures, both chromatographic and ion exchange, and the introduction of many new types of derivatives. The extensive tables of physical constants have been carefully checked against the literature, and several hundred new compounds have been added, including a number of fluoro-organic compounds. The detailed procedures have been tested by experienced workers and by students with limited experience, so that the book can be recommended for use as much in teaching courses as in the wider field of applied organic analysis. Indeed, the only disadvantage of its use as a student text-book is its relatively high cost.

The authors, who pioneered the use of semimicro methods in qualitative organic analysis, have drawn fully from their wide experience in writing this book, with the result that it can be recommended to all interested in this branch of analytical chemistry. R. BELCHER

Publications Received

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Erratum

JULY (1957) ISSUE, p. 527, 1st line. *For* "1·485 g" *read* "1·1481 g."